EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. XIII MARCH, 1959 No. 1

POLYGENES IN EYE PIGMENT PRODUCTION IN DROSOPHILA SPECIES

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Received February 28, 1958

During previous investigations on eye pigments in Drosophila species (Nolte, 1958), it was found that wild type South African strains of *D. melanogaster* and American strains of *D. pseudoobscura* possessed significantly different amounts of the red and the brown eye pigments, and it was postulated that two series of polygenes were segregating in these populations, and affecting the total amounts of the two pigments, respectively. In order to study this problem further and to test the effect of segregation of these genes, a series of investigations was instituted on outcrossing and inbreeding.

(1) Inbred strains of *D. melanogaster* from a previous investigation (Nolte, 1958) were crossed, i.e., Inhaca-3 × Inhaca-1, Inhaca-3 × Inhaca-2, and Inhaca-3 × Graaff-Reinet-2. From the F_2 of each of these crosses fifteen fertilized females were taken and inbred for a further ten generations. From the F_{12} ten of the inbred lines were taken at random from each of the three groups of fifteen and these were tested quantitatively for eye pigments. Seven of these lines were continued to the F_{32} and then again tested quantitatively.

(2) Fourteen fertilized females of *D. melanogaster* from a single collection bottle taken at Stanford Lake in the Northern Transvaal were taken at random from the F_2 and inbred for a further ten generations. Each line was, however, subdivided at the F_3 into either two or three sublines. The F_{12} of each of these lines was tested quantitatively for eye pigments.

(3) From four collection bottles of *D. melanogaster* taken around a rubbish dump at a camp near the Limpopo River in the Northern Transvaal, twelve strains were inbred to the F_9 and then tested quantitatively for eye pigments. A similar study was made of six strains of *D. simulans,* derived from two collection bottles from the same locality.

(4) Six strains of *D. melanogaster,* each from a different collection in the forest, mangrove swamps and around the laboratory of the Marine Biological Station on Inhaca Island, Delagoa Bay, were inbred to the F_7, and then tested for eye pigment content. A similar study was made of six inbred strains of *D. simulans,* derived from three collection bottles taken in the same locality.

TABLE 1. *Relative amounts of red and brown pigments in the eyes of inbred strains from three outcrosses between inbred South African strains of* D. melanogaster

Strain	Inbred line	Red pigment	Brown pigment
Inhaca-1		0.7107 ± 0.0162	0.1552 ± 0.0028
Inhaca-2		1.1577 ± 0.0128	0.1040 ± 0.0015
Inhaca-3		0.9014 ± 0.0090	0.1016 ± 0.0011
Graaff-Reinet-2		1.1321 ± 0.0182	0.1227 ± 0.0022
Inhaca-3 × Inhaca-1	1	0.9812 ± 0.0136	0.1254 ± 0.0012
	2	0.9563 ± 0.0187	0.1308 ± 0.0039
	3	0.8844 ± 0.0166	0.1424 ± 0.0038
	4	0.8312 ± 0.0069	0.1061 ± 0.0013
	5	0.7906 ± 0.0231	0.1242 ± 0.0011
	6	0.7891 ± 0.0065	0.1129 ± 0.0054
	7	0.6646 ± 0.0202	0.1396 ± 0.0030
	8	0.6219 ± 0.0179	0.1327 ± 0.0079
	9	0.6109 ± 0.0316	0.1232 ± 0.0042
	10	0.5797 ± 0.0054	0.1232 ± 0.0023
Mean		0.7710 ± 0.0461	0.1261 ± 0.0035
Inhaca-3 × Inhaca-2	11	1.1437 ± 0.0374	0.0985 ± 0.0018
	12	1.1131 ± 0.0272	0.1219 ± 0.0021
	13	1.0737 ± 0.0330	0.1339 ± 0.0038
	14	0.9854 ± 0.0181	0.1336 ± 0.0036
	15	0.9720 ± 0.0131	0.1211 ± 0.0006
	16	0.9563 ± 0.0227	0.1126 ± 0.0026
	17	0.9021 ± 0.0247	0.1003 ± 0.0046
	18	0.7853 ± 0.0247	0.1058 ± 0.0030
	19	0.7833 ± 0.0204	0.1155 ± 0.0038
	20	0.7750 ± 0.0162	0.1143 ± 0.0023
Mean		0.9490 ± 0.0418	0.1158 ± 0.0039
Inhaca-3 × Graaff-Reinet-2	21	1.0833 ± 0.0179	0.1408 ± 0.0028
	22	1.0791 ± 0.0366	0.1324 ± 0.0025
	23	1.0375 ± 0.0100	0.1406 ± 0.0014
	24	1.0250 ± 0.0208	0.1420 ± 0.0025
	25	1.0167 ± 0.0203	0.1557 ± 0.0046
	26	0.9896 ± 0.0256	0.1374 ± 0.0020
	27	0.9146 ± 0.0255	0.1085 ± 0.0014
	28	0.8813 ± 0.0145	0.1403 ± 0.0047
	29	0.7226 ± 0.0276	0.1310 ± 0.0031
	30	0.6667 ± 0.0347	0.1231 ± 0.0021
Mean		0.9416 ± 0.0462	0.1352 ± 0.0040
After a further 20 generations of inbreeding (S = significant difference) between F_{12} and F_{32}			
	2	0.9338 ± 0.0160	0.1553 ± 0.0046 S
	5	0.8414 ± 0.0193	0.1357 ± 0.0036 S
	10	0.6709 ± 0.0283 S	0.1536 ± 0.0025 S
	11	1.0167 ± 0.0190 S	0.1168 ± 0.0043 S
	12	1.0032 ± 0.0111 S	0.1383 ± 0.0011 S
	19	0.7768 ± 0.0166	0.1083 ± 0.0030
	25	1.0578 ± 0.0221	0.1437 ± 0.0032

(5) To test the segregation of these polygenes in eye mutant strains, the mutants w^{e2} and w^{a3} of *D. melanogaster* were crossed with four of the inbred strains of the first investigation. The mutant color types were extracted from the F_2 of these crosses and inbred for twenty generations and then tested for eye pigment content.

The quantitative estimations of the red and brown pigments were made by means of the photometric measurement of eye extracts, as in previous investigations.

The relative amounts of red and brown pigments in the eyes of the various inbred strains are given in tables 1–6; the amounts are given as extinction (E) for 10 heads per cc of AEA in which the red pigment is extracted and of AMA in which the brown pigment is subsequently extracted. The quantitative estimations are made at 480 mμ for the red pigment and 444 mμ for the brown pigment.

TABLE 2. *Relative amounts of red and brown pigments in the eyes of inbred strains from 14 females of* D. melanogaster, *collected at Stanford Lake*

Strain	Red pigment	Brown pigment
1–1	0.9694 ± 0.0078	0.0863 ± 0.0007
1–2	0.9917 ± 0.0250	0.1220 ± 0.0012
1–3	0.9833 ± 0.0097	0.1031 ± 0.0032
2–1	1.1416 ± 0.0082	0.1159 ± 0.0012
2–2	1.1000 ± 0.0256	0.1062 ± 0.0024
2–3	1.0639 ± 0.0113	0.1130 ± 0.0055
3–1	0.9333 ± 0.0328	0.1225 ± 0.0120
3–2	1.0444 ± 0.0241	0.1050 ± 0.0043
4–1	1.0811 ± 0.0360	0.1042 ± 0.0040
4–2	1.0750 ± 0.0363	0.1129 ± 0.0034
4–3	1.0896 ± 0.0213	0.1251 ± 0.0058
5–1	1.0450 ± 0.0129	0.0746 ± 0.0028
5–2	1.1824 ± 0.0093	0.1109 ± 0.0043
5–3	1.0611 ± 0.0201	0.0887 ± 0.0010
6–1	1.0649 ± 0.0269	0.0934 ± 0.0020
6–2	1.0788 ± 0.0272	0.0929 ± 0.0021
7–1	1.1667 ± 0.0193	0.1138 ± 0.0030
7–2	1.2304 ± 0.0179	0.1015 ± 0.0066
8–1	1.1028 ± 0.0073	0.1198 ± 0.0057
8–2	1.1000 ± 0.0091	0.1204 ± 0.0042
9–1	1.0183 ± 0.0122	0.1126 ± 0.0010
9–2	1.0203 ± 0.0320	0.1122 ± 0.0038
10–1	1.1778 ± 0.0099	0.1126 ± 0.0043
10–2	1.2167 ± 0.0080	0.1021 ± 0.0010
11–1	1.0816 ± 0.0153	0.1150 ± 0.0041
11–2	0.9806 ± 0.0101	0.0997 ± 0.0030
12–1	1.1895 ± 0.0390	0.1216 ± 0.0050
12–2	1.2287 ± 0.0300	0.1107 ± 0.0021
13–1	0.9944 ± 0.0227	0.1108 ± 0.0046
13–2	1.1667 ± 0.0167	0.1135 ± 0.0035
14–1	1.1556 ± 0.0640	0.1196 ± 0.0064
14–2	1.0583 ± 0.0144	0.1213 ± 0.0011
Mean	1.0873 ± 0.0142	0.1089 ± 0.0021

DISCUSSION

During a previous investigation (Nolte, 1958) it became evident that South African wild strains of *D. melanogaster* are genetically heterogeneous for various combinations of two series of genes which affect, respectively, the content of the red and brown eye pigments. The results of outcrossing inbred strains and inbreeding wild strains in the present study confirm the conclusion that these genes are segregating in various populations. The several lines of attack on the problem have yielded the following results.

(1) In the first experiment (table 1) three outcrosses were made with four strains which has been inbred for many years. From these crosses the lines inbred for ten generations showed distributions of pigment content round means which are generally intermediates of the values for the parental strains. As shown by the magnitudes of the standard errors of the means for each of the three series, there is a wide range of variation in red pigment content for all three series of inbred lines; seventeen of the thirty lines are significantly different from the means of their series. For the brown pigment only eleven of the lines are significantly different from the means of their series. On the other hand, after a further twenty generations of inbreeding only three of the seven lines tested had further deviated significantly in their red pigment content while at least five of the lines had deviated significantly in brown pigment content. The ranges of variation and the changes during continued inbreeding provide further evidence for the action of fairly large numbers of genes and for the dif-

TABLE 3. *Relative amounts of red and brown pigments in the eyes of inbred strains from 12 females of* D. melanogaster, *collected at Limpopo River*

Strain	Red pigment	Brown pigment
1	1.1063 ± 0.0203	0.0899 ± 0.0033
2	1.1063 ± 0.0134	0.1186 ± 0.0054
3	1.1053 ± 0.0189	0.1020 ± 0.0018
4	1.0667 ± 0.0253	0.1126 ± 0.0059
5	1.0792 ± 0.0239	0.1301 ± 0.0079
6	1.1029 ± 0.0119	0.1188 ± 0.0062
7	0.9889 ± 0.0241	0.1246 ± 0.0068
8	1.0167 ± 0.0253	0.1100 ± 0.0035
9	1.0057 ± 0.0060	0.1124 ± 0.0070
10	1.1944 ± 0.0201	0.1532 ± 0.0027
11	1.0917 ± 0.0443	0.1194 ± 0.0115
12	1.1515 ± 0.0109	0.1209 ± 0.0077
Mean	1.0846 ± 0.0174	0.1177 ± 0.0045

ferential affect on the two pigments. In these populations and strains more genes for red pigment content are segregating than genes for brown pigment content.

(2) In the second experiment (table 2) the ranges of variation are not so wide, yet with thirteen of the thirty-two substrains deviating significantly from the mean in red pigment, six in brown pigment and three in both. The separation into the various sublines at the F_3 shows continued segregation after the F_2 in lines 2, 7, 13 and 14 for the red pigment, in lines 1 and 4 for the brown pigment, and in lines 3, 5, 10, 11 and 12 for both pigments. In this single collection from a small population a number of genes have segregated into the various sublines.

(3) In the third experiment (table 3) inbred strains were obtained from four locations not well separated, around a camp in the bushveld. Strains 1–8 were derived from the same collection bottle and show no great variation, the only significant deviations from the mean occurring in one strain for the red pigment and in two strains for the brown pigment. Strains 10 and 11 were derived from a different location but from the same collection bottle: strain 10 differs greatly in the content of both pigments.

In the fourth experiment (table 4) strains 1–3 were derived from widely separated locations in the forest, mangrove swamps and the seashore of the island: these three strains differ significantly in the amounts of both pigments. Strains 4–6 were derived from separate collection bottles near the laboratory, and close to the location of strain 3: these strains show no great deviation from each other or from strain 3 in the amounts of both pigments.

It appears that variability in genotype for the genes presumed to condition the pigments quantitatively may be found in any small population of *D. melanogaster,* but that ecological isolation may result in big deviations in the mean genotype.

(4) In the fifth experiment (table 5) the various outcrosses of the two mutants w^{e2} and w^{a3} with some of the inbred strains of experiment 1, have through recombination caused quite considerable changes in the amounts of pigments present in these mutants. For the brown pig-

TABLE 4. *Relative amounts of red and brown pigments in the eyes of inbred strains from 6 females of* D. melanogaster, *collected on Inhaca Island*

Strain	Where collected	Red pigment	Brown pigment
1	Forest	1.0979 ± 0.0250	0.1200 ± 0.0031
2	Mangrove swamp	1.0157 ± 0.0265	0.0908 ± 0.0018
3	Near native hut	1.1000 ± 0.0231	0.1008 ± 0.0033
4	Near laboratory	1.1150 ± 0.0363	0.1094 ± 0.0047
5	Near laboratory	1.0575 ± 0.0276	0.1025 ± 0.0050
6	Near laboratory	1.0333 ± 0.0209	0.0890 ± 0.0044
Mean		1.0699 ± 0.0166	0.1021 ± 0.0048

TABLE 5. *Relative amounts of red and brown eye pigments in the eyes of inbred strains of the mutants* w^{e2} *and* w^{a3} *of* D. melanogaster *after outcrossing with wild Inhaca strains (in table 1)*

Strain		Red pigment	Brown pigment
Inhaca	5	0.7906 ± 0.0231	0.1242 ± 0.0011
	11	1.1437 ± 0.0374	0.0985 ± 0.0018
	19	0.7833 ± 0.0204	0.1155 ± 0.0038
	25	1.0167 ± 0.0203	0.1557 ± 0.0046
w^{e2}	Old stock	0.0616 ± 0.0011	0.0506 ± 0.0005
	ex Inhaca 5	0.0486 ± 0.0016	0.0439 ± 0.0013
	ex Inhaca 11	0.0272 ± 0.0003	0.0371 ± 0.0010
	ex Inhaca 19	0.0356 ± 0.0007	0.0420 ± 0.0015
	ex Inhaca 25	0.0438 ± 0.0011	0.0448 ± 0.0011
w^{a3}	Old stock	0.0542 ± 0.0008	0.0091 ± 0.0003
	ex Inhaca 11	0.0476 ± 0.0016	0.0116 ± 0.0004
	ex Inhaca 19	0.0508 ± 0.0020	0.0111 ± 0.0004
	ex Inhaca 25	0.0560 ± 0.0019	0.0129 ± 0.0006

ment there appears to be a more or less direct proportionality between the modified amounts in the inbred extracted strains and the amounts in the wild strains, but for the red pigment of extracted strains of both alleles the lowest content is present in strains from outcrosses with the wild strain containing the highest amount of red pigment. The variation in pigment content in the strains of extracted mutants, however, prove conclusively the existence of two series of modifiers which condition the total amounts of red and brown pigments in the eyes of *D. melanogaster.* It also shows that standardization of the genetic background is essential in the study of the quantitative effects of different mutant eye-color genes.

(5) In a previous investigation (Nolte, 1958) South African wild strains of *D. simulans* were not observed to show the

TABLE 6. *Relative amounts of red and brown pigments in the eyes of inbred strains of* D. simulans, *collected at Limpopo River and on Inhaca Island*

Strain		Red pigment	Brown pigment
Limpopo	1	1.0271 ± 0.0091	0.1312 ± 0.0035
	2	1.2028 ± 0.0134	0.1240 ± 0.0096
	3	1.2167 ± 0.0245	0.1404 ± 0.0018
	4	1.1528 ± 0.0184	0.1356 ± 0.0058
	5	0.9611 ± 0.0343	0.1214 ± 0.0090
	6	1.1000 ± 0.0246	0.1139 ± 0.0082
Mean		1.1101 ± 0.0318	0.1274 ± 0.0041
Inhaca	1	1.1639 ± 0.0085	0.1052 ± 0.0065
	2	1.1909 ± 0.0057	0.1008 ± 0.0041
	3	0.9600 ± 0.0156	0.1086 ± 0.0046
	4	1.1221 ± 0.0328	0.1162 ± 0.0024
	5	1.0083 ± 0.0083	0.1093 ± 0.0031
	6	1.0400 ± 0.0154	0.0852 ± 0.0019
Mean		1.0825 ± 0.0373	0.1042 ± 0.0043

amount of quantitative variation in pigment as was found for *D. melanogaster*. In table 6 two series of inbred strains of this species, however, show a similar type of variation. Of the Limpopo strains numbers 1–5 were derived from a single collection bottle and of these four show significant deviations from the mean in the amount of red pigment, and one in the amount of brown pigment. Of the Inhaca strains numbers 1–4 were derived from the same collection bottle, and of these three show significant deviations from the mean in the amount of red pigment; strain 6 was derived from a different collection bottle and shows significant deviation in the brown pigment content. The two series differ significantly in the mean amount of brown pigment. In this species, therefore, a similar two series of modifiers seem to be influencing the two pigments quantitatively and differentially. Similar results were obtained in the study of wild strains of *D. pseudoobscura* (Nolte, 1958), and the occurrence of this type of polygenic heredity thus seems to occur generally in wild populations of *Drosophila*.

If the eye pigments are regarded as by-products of metabolism, it may be predicated that these modifiers or polygenes have only a secondary effect on the pigments, but that their main effect lies deeper.

(6) In general it was found that big differences may occur between inbred lines of wild *D. melanogaster* in the amounts of red and brown eye pigments. Robertson and Forrest (1957) determined that in this species the pteridine, isoxanthopterin, which occurs in the biochemical pathway leading to the production of the red eye pigment, shows substantial differences in amount in wild stocks and inbred lines, and state that many gene differences are probably involved. In the present study the first experiment gives coefficients of variation for the red pigment content of the three series which range from 14% to 20%, while that of the brown pigment range round 9%. Possibly a greater number of polygenes for the red pigment were segregating than for the brown pigment. In the Stanford Lake series the coefficients are 7% and 11%, respectively, and for the Limpopo River series 5% and 13%, respectively. The wild populations studied (Nolte, 1958) had coefficients of 13% for the red and 10% for the brown pigment. In certain populations, therefore, the number of genes segregating for the brown pigment may be greater than those for the red pigment, but generally the red series seems to include a greater number of genes than is found in the brown series.

To gain some conception of the number of genes in each of these series, as found occurring in the inbred strains of *D. melanogaster* studied in the present and a previous (Nolte, 1958) investigation, the amounts of red and brown pigments were graphically represented and the frequencies examined for the range and amount of clustering. In the case of the brown pigment the groupings are quite clear, with definite gaps between classes; the mid-points of these groupings or classes are given in table 7, together with the frequencies. If these classes represent the possible combinations of the polygenes of the brown series, and if these genes have approximately similar, and cumulative, effects, the 9 classes could indicate the action of 8 pairs of genes. For the red pigment the clusters or groupings are not quite as definite and two possible frequency distributions are given in table 7, with the second showing more clearcut gaps than the first. If the same conclusion is drawn for the red pigment as for the brown, it seems that at least eight, possibly eleven, pairs of polygenes are found in the wild populations and inbred strains studied.

It should be noted that a number of strains included in this summation have not been inbred for a sufficient number of generations; if the red series includes a larger number of genes than the brown series, the less clearcut distribution fre-

TABLE 7. *The frequencies of 103 inbred lines of* D. melanogaster *for different amounts of red and brown eye pigments*

Brown pigment											
Class mid-points	0.073	0.083	0.093	0.103	0.113	0.123	0.133	0.143	0.153		
Frequency	1	2	8	21	28	25	9	6	3		
Red pigment											
Class mid-points	0.60	0.66	0.72	0.78	0.84	0.90	0.96	1.02	1.08	1.14	1.20	1.26
Frequency	4	2	2	5	1	7	13	16	36	10	6	1
Class mid-points	0.53	0.62	0.71	0.80	0.89	0.98	1.07	1.16	1.25		
Frequency	1	3	4	7	7	24	40	14	3		

quency for the red pigment might be ascribed to the fact that some of these strains were not sufficiently homozygous for these genes.

This system of polygenic heredity may, however, be accounted for under a second hypothesis. The quantitative effects may result from secondary, pleiotropic, expressions of the major eye-color genes, additional to their main functions. The range of quantitative variation could then be ascribed to the presence of wild type isoalleles with differing potency, in the sense of differences in the quantity of end product. Such isoalleles have been found in *D. melanogaster,* e.g., at the ci locus (Stern and Schaeffer, 1943). The quantitative effect of such isoalleles may be comparable with the effect of mutant genes at these loci. In a series of investigations (Nolte, 1952, 1954, 1955) fifteen mutant loci have been found to reduce mainly the amount of red pigment, five mainly the amount of brown pigment, and twelve the amounts of both pigments. To account for the range of quantitative variation found in natural populations and inbred lines from these, it would be necessary to postulate the presence of isoalleles at a fairly large number of these loci; common occurrence of wild type isoalleles has not been demonstrated.

An extension to the polygenic concept could be that the quantitative eye pigment effects are secondary to some other quantitative main effects of these genes, in which case the primary effects must also be undergoing segregation and recombination in the various wild populations: such effects have not been noted.

No population examined has proved to be homozygous for these genes with quantitative effect, although various small populations have been shown to possess greater heterogeneity for either the alleles of the red series or those of the brown series. Phenomena such as isolation and genetic drift may be expected to result in big deviations in average genotype, from year to year and from locality to locality. Whether any selective advantage appertains to these series of genes is not demonstrated, since no trend has been noted in correlation with different climatic conditions and habitats.

SUMMARY

Inbred lines of wild South African populations of *D. melanogaster,* established from the F_2 of females collected in geographically separated areas and from outcrosses from inbred strains, have been investigated for the amounts of red and brown eye pigments.

(1) Inbred lines obtained from outcrosses between inbred strains differing widely in their content of red and brown pigments, showed segregations for pigment content ranging round means which were generally intermediate to the amounts in the parental strains. The ranges of variation and further changes during con-

tinued inbreeding demonstrate the action of fairly large numbers of genes, and the differential effect on the two pigments.

(2) Single collections from small populations show segregations into several sublines for pigment content.

(3) Collections from well separated locations have yielded inbred lines which differ significantly in the content of both pigments, while for collections close together in the same area no great deviations were shown in inbred lines.

(4) Outcrossing of the mutants w^{e2} and w^{a3} of *D. melanogaster* to inbred wild type lines with different amounts of eye pigment, showed after extraction and inbreeding that strains of these mutants may be developed with greatly different amounts of both pigments.

(5) Similar results were obtained with *D. simulans* collected in two widely separated areas, there being mainly intra-group deviations in the amount of red pigment and inter-group deviations in the brown pigment content.

(6) In *D. melanogaster* frequency distributions for the amounts of the two pigments in 103 inbred lines show definite clustering in the case of the brown pigment and a range of nine classes. In the case of the red pigment the clustering is not quite as definite but distributions with twelve or nine classes are obtained. If these classes represent the effects of the segregation of polygenes with approximately similar, and cumulative, effects, the brown series could be represented by eight pairs of alleles and the red series by eight to eleven pairs of alleles.

(7) Various alternatives to the polygenic hypothesis are discussed, and it is noted that while isolation and genetic drift may cause big temporal and spatial differences in average genotype, no sign of selective advantage has been noted for these genes.

Literature Cited

Nolte, D. J. 1952. The eye-pigmentary system of Drosophila. III. The action of eye-colour genes. J. Genet., **51**: 142–186.

——. 1954. The eye-pigmentary system of Drosophila. IV. The pigments of the vermilion group of mutants. J. Genet., **52**: 111–126.

——. 1954. The eye-pigmentary system of Drosophila. V. The pigments of the light and the dark groups of mutants. J. Genet., **52**: 127–139.

——. 1955. The eye-pigmentary system of Drosophila. VI. The pigments of the ruby and red groups of mutants. J. Genet., **53**: 1–10.

——. 1958. Eye pigment relationships in three species groups of Drosophila. Evolution, **12**: 519–531.

Robertson, F. W., and H. S. Forrest. 1957. Genetic variation of isoxanthopterin content in *Drosophila melanogaster*. Univ. Texas Publ., 5721: 229–237.

Stern, C., and E. W. Schaeffer. 1943. On wild isoalleles in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Wash., **29**:361–367.

LATE CENOZOIC EVOLUTION OF THE SIERRAN BIGTREE FOREST

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Received March 27, 1958

INTRODUCTION

Bigtree (*Sequoiadendron giganteum*), which ranges discontinuously from the northern to the southern Sierra Nevada in California (fig. 1), is the last relict of a phylad that was vigorous during the Late Mesozoic and Early Tertiary. The bigtrees of Cretaceous and Paleocene-Eocene times (*S. reichenbachi, S. couttsiae*) differ sufficiently in their morphology from *S. giganteum,* however, to show that they are not immediately ancestral to the modern species. Furthermore, most of the plants that lived with these older bigtrees are quite different from those that make up the forest in which *Sequoiadendron* now occurs.

The oldest fossil bigtree communities now known that can be considered closely ancestral to the living are of Late Miocene and Early Pliocene age. Not only are the foliar and reproductive structures of the Late Tertiary bigtree (*S. chaneyi*) very similar to those of the modern forest goliath, but most of its fossil associates can scarcely be distinguished from those in the present community. These Late Tertiary forests characterized by bigtree occur at four localities in western Nevada (fig. 1). They are represented by the Aldrich Station, Fallon, and Middlegate floras of Late Miocene age (Axelrod, 1956),[1] and by the Chalk Hills flora of Early Pliocene age (Axelrod, MS). The topographic setting under which these floras lived, the composition of the forests, and the paleoecology at the several sites have been discussed in detail. The gradual evolution of the modern bigtree forest from the Late Tertiary community, a topic not previously considered, is outlined here in a provisional way. In order to discuss the Late Cenozoic history of the forest, it is necessary first to note briefly the nature of the living community.

[1] These floras have earlier been considered Mio-Pliocene in age (Axelrod, 1956); for convenience they are referred to here as Late Miocene.

THE MODERN FOREST

Bigtree has a discontinuous occurrence at middle altitudes in the Sierra Nevada. Locally it forms pure communities, but more commonly is mixed in with the typical dominants of the mid-Sierran forest, notably yellow pine, white fir, sugar pine, incense cedar, and (at the north) Douglas fir. The stands are generally small, and only two are estimated to have covered more than 10 square miles; at the advent of white man the total area occupied by the community probably was not much more than 50 square miles. The forest may be relatively open in broad swales, but in valleys, narrow canyons, or on slopes it usually has a dense understory of small trees and shrubs. These include bigleaf maple, white alder, mountain alder, dogwood, black oak, green manzanita, deerbrush, serviceberry, chinquipin, hazelnut, azalea, and others listed below (under Composition, *see* Sierra-Cascade Component).

Bigtree occurs chiefly in sites that represent optimum conditions for growth of the pine-fir forest of which it is an integral part. This is shown by the great size—commonly 200 feet tall, and 8 to 10 feet in diameter—that yellow pine, sugar pine, white fir, and the other conifers attain in areas where they are associated

with bigtrees, yet even they are dwarfed by these forest giants. Bigtrees live most commonly on flats and slopes where there is a rich, deep, well-drained soil. They have a broadly-spreading, but very shallow root system that is bathed by water seepage at all times, either at or just below the surface; in many of the groves the sound of trickling water is present even at the end of the long dry summer

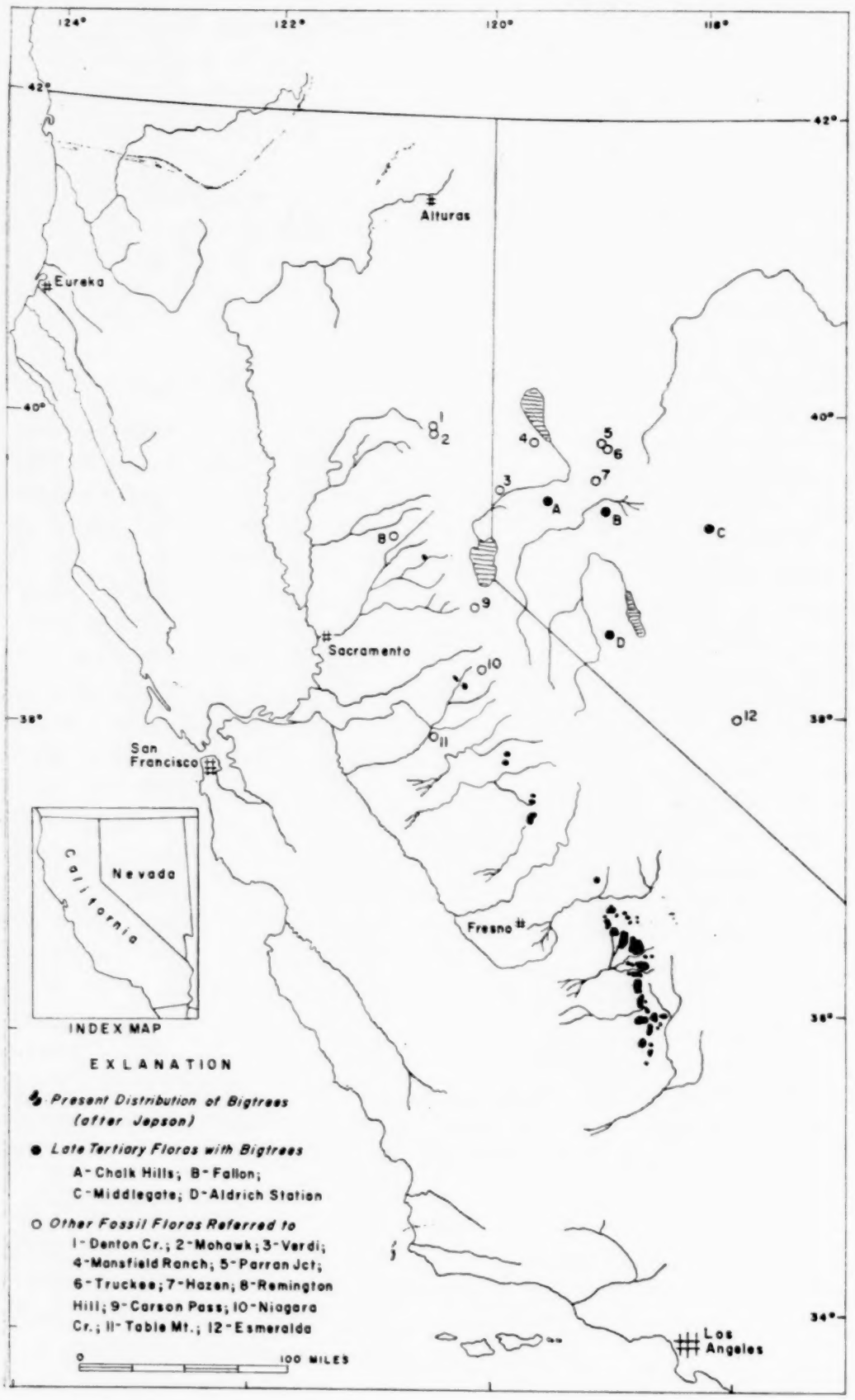

FIG. 1. Occurrences of living and fossil bigtrees in the far West, and floras to which reference is made.

season. Since it is so closely associated with water, as are many of its regular associates such as alder, azalea, dogwood, maple, and willow, the bigtree groves essentially represent a hydrophytic community of Sierran forest.

Bigtree has an altitudinal range that corresponds closely with that of the yellow pine forest. In the southern sector the lowest groves occur in deep valleys with cool air drainage, and in proximity to oak woodland and chaparral. In such sites precipitation is approximately 25 to 30 inches yearly. Over most of its area the groves are in the middle part of the yellow pine forest, with annual rainfall exceeding 60 inches at the north, and decreasing to 35–40 inches at the south; snowfall is heavy throughout the region. The highest groves range up to approximately 7500 feet, where they are in protected sites at the lower margin of fir forest. Summers are warm but not hot, with extremes rarely exceeding 85° F. Winters are not excessively cold, with extremes only occasionally falling below 0° F, and then for but brief intervals.

The Late Tertiary Forest

Many fossil plants that are scarcely distinguishable from species that now live with bigtree have been found with its Late Tertiary relative (*S. chaneyi*) in Nevada. In addition, a number of trees and shrubs whose nearest descendants are not now found in the community also occurred with them. The associates of bigtree differed from locality to locality: the fossil floras display local differences as much as the living, depending on site, altitude, and other factors. In general, the fossil bigtree communities of Nevada comprised two facies. In the region from 60 to 120 miles east of the Sierra Nevada, the Fallon, Aldrich Station, and Middlegate floras show that bigtree and its associates inhabited cooler, moister valleys bordering the sites of deposition which were dominated by live oak woodland and chaparral that survived under 25 inches yearly rain (Axelrod, 1956). Analogous environments occur today in the southern Sierra Nevada near the lower margin of forest, as at Clough Cave on the South Fork of the Kaweah River and near Camp Nelson on the Tule River. On the other hand, the Chalk Hills flora from the east margin of the Early Pliocene Sierra Nevada represents a humid phase of the bigtree forest much like that in the moister parts of the Sierra, in areas where oak woodland and chaparral are rare, and where rainfall is 35 inches or more. Clearly, the species were mixed in a ecotone of subhumid climate in west-central Nevada, and of humid climate in western Nevada.

To analyze the evolution of the modern from the Tertiary community, the differences between them must now be considered. This can best be done by grouping the plants into floristic units, termed elements and components, which express their historical relations in terms of the history of the Arcto-Tertiary and Madro-Tertiary Geofloras in western North America (Chaney, 1944; Axelrod, 1950a). The general distribution of most of the living species related to those that made up the floristic units of these geofloras which formed the Late Tertiary bigtree forest and its border communities is outlined in figure 2.

Arcto-Tertiary Geoflora

This temperate forest, characterized by deciduous hardwoods and conifers, occurred largely at higher latitudes in the Early Tertiary (Chaney, 1938; 1948; Chaney & Hu, 1940). In response to a trend toward lowered temperature following the Eocene, it shifted southward into the region of the Columbia Plateau and northern Great Basin during the Oligocene, and was well established in central Nevada by Miocene time. The following floristic units of this geoflora contributed to the Late Tertiary bigtree forest.

West American Element. Represented in this group are fossil species whose nearest modern descendants are in the western conifer forests. Three major

FIG. 2. Distribution of modern vegetation in the far West corresponding to floral elements which comprised the Late Tertiary bigtree community. Relations with Eastern Asia and Eastern North America discussed in text.

subdivisions, or components, are recognized.

1. Sierra-Cascade Component. This group includes those fossil plants whose closest living relatives make up the forests of the Sierra Nevada and southern Cascades, the higher Coast Ranges, and the mountains of southern California. The following species which are represented in the Late Tertiary bigtree forests of

Nevada have descendants in the living community:

Fossil species	Related living species
Abies concoloroides	*A. concolor*
Acer columbianum	*A. glabrum*
Acer oregonianum	*A. macrophyllum*
Alnus smithiana	*A. tenuifolia*
Amelanchier carsoniana	*A. alnifolia*
Arctostaphylos patuloides	*A. patula*
Castanopsis mulhollandana	*C. sempervirens*
Ceanothus chaneyi	*C. intergerrimus*
Mahonia reticulata	*M. aquifolium-repens*
Pinus florissanti	*P. ponderosa*
Pinus wheeleri	*P. lambertiana*
Pseudotsuga sonomensis	*P. taxifolia*
Populus eotremuloides	*P. trichocarpa*
Populus pliotremuloides	*P. tremuloides*
Prunus moragensis	*P. emarginata*
Rhododendron nevadensis	*R. occidentale*
Ribes stanfordianum	*R. nevadense*
Salix hesperia	*S. lasiandra*
Salix knowltoni	*S. lemmonii*
Salix wildcatensis	*S. lasiolepis*
Sequoiadendron chaneyi	*S. giganteum*

The conifers in this list include species similar to all the forest dominants that occur with bigtree today, except for incense cedar (*Libocedrus decurrens*) which is still unrecorded in these floras. All the others represent species whose living relatives are common members of the bigtree community.

Certain trees and shrubs in the living bigtree forest are not now known to have counterparts in the Late Tertiary forest of Nevada. Some of them have been recorded elsewhere in the Middle and Late Tertiary floras of the far West, and the discovery of new fossil floras in western Nevada may show that they were represented there with bigtree. Among the more important of these plants are:

Fossil species	Related living species
Alnus hollandiana	*A. rhombifolia*
Arctostaphylos verdiana	*A. nevadensis*
Chamaebatia prefoliolosa	*C. foliolosa*
Cornus latahense	*C. nuttallii*
Cornus ovalis	*C. californica*
Libocedrus masoni	*L. decurrens*
Quercus pseudolyrata	*Q. kelloggii*
Salix boisiensis	*S. scouleriana*
Symphoricarpos salmonensis	*S. albus*

In addition, there are other common species in the bigtree community that are not now known to have a fossil record, including:

Calycanthus californica	*Prunus subcordata*
Ceanothus cordulatus	*Rubus parvifolius*
Corylus californica	*Taxus brevifolia*

All of them probably had close relatives in the bigtree forest of Nevada, and future collections may disclose their presence there.

On the other hand, some species of the Sierra-Cascade Component that have been recorded with fossil bigtree are related to plants that live now at lower, warmer levels in the Sierran forest, below the bigtree groves. These include *Acer minor* (*negundo*),[2] *Fraxinus coulteri* (*oregona*), and *Torreya nancyana* (*californica*). Others, however, such as *Ceanothus leitchii* (*velutinus*) and *Pinus wheeleri* (*monticola*)[3] have their nearest related species at higher altitudes in the Sierra Nevada, or else in the Sierra-Cascade forest to northward, such as *Picea sonomensis* (*breweriana*) and *Pachystima nevadensis* (*myrsinites*).[3]

2. Coast Component. Species of this group have living descendants in the humid Coast Forest, ranging chiefly from northwestern California into British Columbia. The following occurred with bigtree in western Nevada:

Fossil species	Related living species
Betula thor	*B. occidentalis*
Castanopsis sonomensis	*C. chrysophylla*
Thuja dimorpha	*T. plicata*
Tsuga sonomensis	*T. heterophylla*
Salix owyheeana	*S. hookeriana*

Although typically of coastal occurrence, chinquipin (*Castanopsis*) has a few relict stations in the lower part of the Sierra-Cascade forest in the Sierra Nevada northeast of Placerville.

3. Rocky Mountain Component. This floristic unit includes species that are related to plants which are common in the

[2] Species names in parenthesis indicate living plants closely related to the fossils.

[3] Also common to the Rocky Mountain forest.

Rocky Mountain forests, and some of them range westward into the higher mountains of the Great Basin. The following have been recorded with bigtree in western Nevada:

Fossil species	Related living species
Acer grandidentatum	*A. bolanderi*
Crataegus middlegatei	*C. chrysocarpa*
Populus payettensis	*P. angustifolia*

East America-East Asian Elements. Fossil species comprising these elements have their closest descendants in the eastern parts of the northern continents, areas characterized by ample summer rain. The following contributed to the Late Tertiary bigtree community in western Nevada:

Fossil species	Related living species
Ailanthus indiana	*A. altissima*
Betula thor	*B. papyrifera-japonica*
Betula vera	*B. lenta*
Comptonia parvifolia	*C. asplenifolia*
Platanus dissecta	*P. occidentalis*
Populus washoensis	*P. grandidentata*
Quercus simulata	*Q. myrsinaefolia*
Ulmus moorei	*U. crassifolia*
Zelkova nevadensis	*Z. serrata;* spp.

Madro-Tertiary Geoflora

The northward migration of this geoflora from southwestern North America, where it apparently originated during the Late Cretaceous and Early Tertiary (Axelrod, 1958a), was chiefly in response to the expansion of dry climate. During the Late Miocene and Early Pliocene it overlapped the Arcto-Tertiary Geoflora in western Nevada. Although largely occupying drier sites bordering the forest, some of these plants were closely associated with bigtree, much as their derivatives now occur close to it at lower, warmer altitudes.

California Woodland and Chaparral Elements. Fossil plants comprising these elements are related to species that are typical now of California communities. The following fossil species have close modern descendants that are still found within the shade of bigtree:

Fossil species	Related living species
Cercocarpus antiquus	*C. betuloides*
Quercus hannibali	*Q. chrysolepis*
Quercus wislizenoides	*Q. wislizenii*
Rhamnus precalifornica	*R. californica*

Other woodland and chaparral species that lived with bigtree have modern counterparts that are found well below it today. Some of them interfinger with the warmer, drier margins of the yellow pine forest, but they live chiefly at lower altitudes or to southward:

Fossil species	Related living species
Aesculus ashleyi	*A. parryi*[4]
Amorpha oblongifolia	*A. californica*
Arbutus matthesii	*A. menziesii*
Ceanothus precuneatus	*C. cuneatus*
Garrya gianellae	*G. fremontii*
Plantanus paucidentata	*P. racemosa*
Salix payettensis	*S. exigua* (group)
Salix truckeana	*S. gooddingii*
Styrax middlegatei	*S. californica*

Sierra Madrean Woodland Element. Fossil plants representing this group have their nearest living relatives in the southwestern United States and adjacent Mexico, a region of summer rainfall. The following occurred with the bigtree community in the Late Tertiary of Nevada:

Fossil species	Related living species
Arbutus prexalapensis	*A. xalapensis-mollis*
Bumelia beaverana	*B. lanuginosa*
Fraxinus alcorni	*F. velutina*[5]
Persea coalingensis	*P. podadenia*
Populus sonorensis	*P. brandegeei*
Robinia californica	*R. neomexicana*

Conifer Woodland Element. Fossil plants of this alliance have close descendants that make up the piñon-juniper woodland of the Great Basin and Colorado Plateau. The following have been re-

[4] Occurs in northern Baja California.

[5] Occurs also in southeastern California.

corded in the Late Tertiary floras that have bigtrees:

Fossil species	Related living species
Amelanchier apiculata	*A. utahensis*
Cercocarpus holmesii	*C. paucidentatus*
Cercocarpus linearifolius	*C. ledifolius*
Fraxinus millsiana	*F. anomala*
Juniperus nevadensis	*J. utahensis*
Symphoricarpos wassukana	*S. oreophila*

Summary

The fossil plants that were associated with bigtree in the Late Tertiary floras of Nevada comprised two divergent groups—one of Arcto-Tertiary, the other of Madro-Tertiary origin. The former chiefly included deciduous hardwoods and conifers of temperate requirements, the latter largely sclerophylls of semiarid relations. Species representing the floristic units of these geofloras, whether element or component, occurred at every site where bigtree has been recorded. All species of both geofloras have survived down to the present in scarcely modified form. Although some comprise part of the living bigtree community, many no longer have close equivalents represented in it. This brings us to an explanation of the derivation of the modern from the ancestral Late Tertiary forest.

Floristic Evolution

The principles governing an analysis of the evolution of plant communities were synthesized first by Good (1931), and then modified and elaborated by Mason (1936; 1947) and Cain (1944). During the past two decades these principles have been applied generally to studies of the evolution of Tertiary vegetation in the western United States (for example, *see* Axelrod, 1939, 1940b; 1948; 1950d; 1958a; Chaney, 1938; 1948; 1958; MacGinitie, 1953; Mason, 1934). As Mason (1947) has observed, floristic evolution must be interpreted from the interactions of (1) the environment, chiefly climate, in terms of its spatial variation and changes in time, (2) the physiology of plants in terms of their adjustment to the environment, and (3) the changing genetics of populations with respect to increasing or decreasing genetic diversity as it may affect the range of variation of tolerances of the species, and the development of new genetic races.

Although it must be granted that some of these aspects of the problem are not easy to interpret when we are dealing with fossil floras, reasonably sound inferences can nonetheless be made with respect to all of them. For example, the regional changes of climate can be reconstructed satisfactorily by analyzing a series of contemporaneous floras over a broad area according to the principles of paleoecology (Clements, 1916; Chaney, 1938; Cain, 1944). Secular climatic change can be interpreted from sequences of successively younger floras, or cliseres, in local areas. The physiology of particular taxa, in terms of their adjustment to environment, may be inferred from their associates. For example, the regular Miocene occurrence of *Sequoia* with numerous deciduous hardwood genera that are confined now to areas of ample summer rainfall in eastern North America and eastern Asia suggests that *S. affinis* required ample summer rain in contrast to its nearest descendant (*S. sempervirens*) which persists in summer-dry California.[6] The same reasoning applies to *Lyonothamnus,* for during Miocene time it lived with many genera that occur now in the summer-wet part of northern Mexico, yet the living species survives in a summer-dry area. Different genetic composition of populations is suggested when a fossil species closely similar to a living plant is found with a community which differs widely from that with which its modern counterpart occurs. For example, during the Late Tertiary aspen (*Populus pliotremuloides*) lived with the humid redwood forest at sea level in central California (Axelrod, 1944b), and also with floras dominated by live oak

[6] Its restriction to the coastal fog belt no doubt compensates for the lack of summer rain.

woodland and chaparral vegetation (Axelrod, 1937; 1944a; 1950c), communities that differ greatly from those with which aspen is found today. Such occurrences are relatively common and have led to the suggestion that aspen and many other Late Tertiary species had genetic races, or ecotypes, which are no longer represented in the modern flora (Axelrod, 1941). By contrast, the rapid adaptation of the entire flora of California and bordering areas to the summer-dry climate which developed here only during the Quaternary, suggests that these plants recently have either (1) developed new ecotypes which have been able to survive in this region, or that (2) the Late Pliocene species were preadapted to this unique climate, or that (3) the flora includes a mixture of both.

The gradual evolution of the modern bigtree forest from the Late Tertiary ancestral community thus appears explicable in large measure on the basis of the responses of plants (changing physiologic-genetic relations) to changes in climate that have occurred during the 15 million years which have elapsed since the Late Miocene. It is now well established that following the wide occurrence of moist, tropical climates of the Paleocene and Eocene epochs, there was a secular trend toward increased continentality. During succeeding epochs there was a gradual reduction in total rainfall, and a shift in seasonal occurrence from ample summer moisture early in the period, to its gradual reduction and final elimination at the close of the Tertiary. This was accompanied by an increase in the frequence and amount of winter rain. Paralleling this precipitation trend, there was a gradual development of hotter summers characterized by a high evaporation rate, and winters shifted from mild and frostless early in the Cenozoic to cold at the close of the period.

These successive changes appear to account (1) for the migration of the bigtree community from Nevada to the west Sierran slope during the Pliocene, (2) for the elimination from the Late Tertiary community of a number of plants whose ranges of tolerance for summer rain, or summer heat, or winter cold, were exceeded, (3) for the persistence in the Sierra Nevada of a part of the flora as the new Mediterranean climate evolved, and (4) for the gradual rise to dominance during the later Cenozoic of some plants that formerly had a more subordinate place in the ancestral forest.

Migration

Withdrawal of the Late Tertiary bigtree community from west-central Nevada was due chiefly to the gradual reduction in rainfall. During the Late Miocene, bigtree and its forest associates largely occupied moister slopes and valleys bordering the lowland Nevada basins where oak woodland was dominant. Early Pliocene floras show clearly that by the latter part of that time forest had largely been eliminated from the lowlands to the east of the Sierra. For example, the Esmeralda flora (Axelrod, 1940a) includes oak woodland and chaparral vegetation, with desert-border plants on nearby drier slopes. If forest was in the area it must have been confined to the bordering hills, to sites sufficiently remote from the basin of deposition so that records of it are absent. The late Early Pliocene Parran Junction flora also is dominated by live oak woodland. A few seeds of spruce are represented, but they probably were transported into the lowlands by air and water currents to judge from the fact that needles or twigs are not represented in the collection. These Early Pliocene floras were living to the east of the Sierra Nevada at distances ranging from 50 to 60 miles (fig. 1), when rainfall over the lowlands varied from 15 to 20 inches, as compared with 25 inches in the Late Miocene. Westward, nearer the Sierra Nevada, rainfall gradually increased during the Early Pliocene in much the same way that it does today. Thus, the Mansfield Ranch flora near Pyramid Lake has oak woodland vegeta-

tion, but forest species such as fir and spruce were present in small numbers in this area where precipitation was 25 inches. Farther west, at the edge of the Sierra Nevada, there was sufficient precipitation to support a rich bigtree forest during the Early Pliocene, as shown by the Chalk Hills flora which suggests that rainfall was 35 inches annually.

A further reduction in rainfall over western Nevada is indicated by the Middle Pliocene floras. The Hazen and Truckee floras of this age, situated 40 to 60 miles east of the Sierran front, show that arboreal vegetation was confined chiefly to stream- and lake-borders, and the interfluves supported scattered patches of oak savanna separated by broad grassy plains (Axelrod, 1948, 1950d). The Verdi flora, preserved in a basin in the east Sierran foothills, is dominated by stream-border trees and shrubs. Forest was confined largely to bordering hills, where rainfall was approximately 25 inches (Axelrod, 1958b). The Verdi is much drier than the Chalk Hills flora which lived in the nearby area earlier in the Pliocene, under 35 inches yearly precipitation. These relations further support the inference that in response to the secular trend toward lowered rainfall, the bigtree forest of west-central Nevada was eliminated from the lowlands during the Early Pliocene, and had largely migrated to the moister Sierra Nevada by the Middle Pliocene, the driest part of the Tertiary (Axelrod, 1948).

Fossil floras from the Sierra Nevada demonstrate that this westward shift of the bigtree community was migration in the full sense of the word. It cannot be explained by suggesting that whereas the forest was eliminated from Nevada, it survived on the west slope of the Sierra Nevada where it had occurred contemporaneously in the Miocene. During that epoch the range was dominated by a forest of quite different composition. Miocene floras from the general summit region, as represented by the Mohawk, Carson Pass, and Niagara Creek floras, are typified as deciduous hardwoods, and conifers are largely absent. Among the dominants of these forests were species such as *Diospyros andersonae* (*virginiana*),[7] *Fagus washoensis* (*grandifolia*), *Liquidambar pachyphyllum* (*styraciflua*), *Persea coalingensis* (*podadenia*), *Populus lindgreni* (*heterophylla*), *Nyssa copeana* (*sylvatica*) *Quercus payettensis* (*extinct*), *Pterocarya mixta* (*stenoptera*), and others which show that the range had only a low altitude, and was characterized by moist, warm temperate climate. The somewhat younger Miocene floras show generally similar relations. The Remington Hill flora (Condit, 1944a) from near the lower middle slope includes species related to those now in the coast redwood forest, together with such deciduous hardwoods as *Aesculus preglabra* (*glabra*), *Berchemia multinervis* (*scandens*), *Crataegus newberryi* (*cuneata*), *Juglans pseudomorpha* (*nigra*), *Populus washoensis* (*grandidentata*), *Liquidambar pachyphyllum* (*styraciflua*), *Quercus winstanleyi* (*aleiana*), and *Ulmus californica* (*americana*). Fossil representatives of typical Sierran forest species are unknown. The Table Mountain flora (Condit, 1944b) from the foothill belt has woodland and chaparral vegetation which flanked a valley forest of deciduous hardwoods, including species of *Acer, Berchemia, Carya, Cercis, Cornus, Nyssa,* and *Ulmus,* all of which have their nearest relatives in the eastern United States. The typical conifer dominants of the Sierran forest do not have representatives in the flora. A newly-discovered flora at Denton Creek, near Blairsden in the northern Sierra Nevada, is very similar to the Table Mountain flora, except that chaparral and dry woodland plants are rare. As might be expected from its position 150 miles farther north, and higher in the range, the Denton Creek is more humid than the Table Mountain flora. Late Miocene relatives of Sierran forest species are not recorded at Denton Creek.

[7] Species in parenthesis are living plants most nearly related to the fossils.

Its great similarity to the Table Mountain flora demonstrates that the northern summit region must have been quite low at the close of Miocene time.

The paleoclimates which may be inferred for these floras suggest that winter temperatures were still too mild on the windward flanks of the Sierra Nevada in Late Miocene time for most species of the Sierra-Cascade Component, and particularly for the fossil species of bigtree, yellow pine, white fir, sugar pine, and Douglas fir and their associates that then lived to the east of the range, in west-central Nevada. Only in post-Miocene time, when the climate had become cooler, was it possible for the bigtree forest to migrate from west-central Nevada to the Sierra, occupying first the leeward, and thence the summit and higher windward slopes of the range. Judging from the character of the Middle Pliocene floras, we may infer that the forest was well established in the higher parts of the range by that time, which stood near 1000–2000 feet in the northern, and 3000 feet in the central section (Axelrod, 1957a). The range could not have been much higher otherwise floras to leeward would reflect the presence of a regional rain shadow, and none is evident when cliseres to the east and west of the range are compared critically (Axelrod, 1957b).

Climatic Selection and Segregation

During and following the migration of the bigtree forest to the Sierra Nevada, climate was acting selectively upon the varying tolerances of the species to segregate out the present community. The following statements summarize this aspect of the problem in terms of the probable effects that the disappearance of summer rain, the development of cooler winters, and the appearance of hot dry summers had on the ancestral community.

Summer rain. Gradually diminishing summer rain was largely responsible for the elimination of the Arcto-Tertiary species of *Ailanthus, Betula, Comptonia, Populus* (cf. *grandidentata*), *Quercus* (cf. *myrsinaefolia*), *Ulmus,* and *Zelkova* of the East American and East Asian Element from the forest during the Pliocene. Some of the hardier members of these groups probably survived with *Sequoiadendron* down to the Plio-Pleistocene interval. Not only do species of *Acer, Nyssa, Populus, Ulmus,* and *Zelkova* of these elements occur in the Middle Pliocene floras of central California and Nevada, but *Castanea, Persea, Populus* (cf. *grandidentata*), *Pterocarya, Trapa* and *Ulmus* persisted in central California into the Late Pliocene (Curtis Ranch, Napa, Sonoma, San Joaquin floras). Furthermore, in the Late Pliocene Sonoma floras some of these plants were associated with the Sierra-Cascade Component (Axelrod, 1944b; 1950b), which was not yet wholly segregated from the Coast Component of *Abies, Sequoia, Rhododendron, Tsuga,* and their humid associates.

Species of the Sierra Madrean Element of the Madro-Tertiary Geoflora, represented by descendant types now in the southwestern part of North America, also were eliminated from the Sierran forest and its margins as summer rains were reduced. Since certain members of this group, in genera such as *Ilex, Mahonia, Quercus, Rhus, Robinia* and *Sapindus,* also persisted into the Middle and Late Pliocene in central California and Nevada, some of them probably were still living on the margins of the bigtree forest during this interval, when the community occupied the windward slope of the low Sierran ridge.

The disappearance of summer rain may account also for the extinction of certain fossil ecotypes in the Late Tertiary bigtree, and other communities. Fossil ecotypes appear to be analogous to those of widely distributed living species, such as yellow pine, white fir, Douglas fir, and white pine, all of which display minor regional differences in bark, cone, habit, and other characters in the environments they now occupy, and which respond specifically to the climates of their respective areas. Although the fossils are

not represented by sufficient material to group them into regional races, ecologic relations suggest strongly that in some cases the species probably comprised several races. For example, *Picea sonomensis,* which is allied to *P. breweriana* of the upper part of the Sierra-Cascade forest in the Klamath-Siskyou-Trinity mountain region of northwestern California, occurred with several different forests in the Middle and Late Tertiary: (1) in the Middle and Late Miocene Blue Mountains, Trout Creek, and Thorn Creek floras of Oregon and Idaho it is recorded with other conifers that were associated with numerous deciduous hardwoods of the East American and East Asian Elements (Chaney, 1959); (2) in the Late Miocene floras of west-central Nevada it is recorded with the bigtree forest which was then living close to oak woodland and chaparral vegetation (Axelrod, 1956); (3) in the Early Pliocene Alvord Creek flora of southern Oregon *P. sonomensis* lived with a typical Sierra-Cascade Component, but in proximity to chaparral (Axelrod, 1944c); and (4) in the Late Pliocene Sonoma flora of central California it is recorded at sea level with a humid coast redwood forest (Axelrod, 1944b). Other fossil ecotypes that lived with bigtree included *Thuja dimorpha* (*T. plicata*) and *Tsuga sonomensis* (*heterophylla*) of the Coast Component. Like *P. sonomensis,* both are represented in the Blue Mountains flora with numerous deciduous hardwoods in a humid climate characterized by ample summer rain, they occur in the Late Miocene of western Nevada with bigtree in a subhumid climate, and *Tsuga* is also represented in the Late Pliocene Sonoma flora where it was close to live oak woodland vegetation. We may conclude that ecotypes of species of the Coast and Sierra-Cascade Components comprised ecologic races that were adapted to warmer and drier environments than are their nearest living descendants. Since all of these presumed ecotypes lived under a climate of summer rain down to the end of the Tertiary, at which time they largely disappeared, their extinction probably was due to the elimination of summer precipitation.

Extinction of ecotypes may also have had a role in developing certain differences between the northern and southern parts of the forest. For example, today Douglas fir occurs only in the northern groves which are well up in the moister, cooler parts of the range. Yet in the Late Tertiary *Pseudotsuga sonomensis* occurred not only with the humid phase of the bigtree forest (Chalk Hills flora), but also at its drier margins where it was in ecotone with oak woodland (Aldrich Station flora). The elimination of this tree from the southern half of the Sierra Nevada, and most of the bigtree community, probably was due to the development of a hotter summer climate during the Quaternary following the elimination of summer rain: the ecotype that earlier ranged into such an environment appears to be no longer represented in the modern flora. Extinction of ecotypes may account also for some differences between the lower and upper parts of the forest. Thus, aspen occurs only in the bigtree groves well up in the range at cooler levels, yet during the Late Tertiary *P. pliotremuloides* was associated with woodland and chaparral vegetation at the drier, warmer margins of the forest (Middlegate, Aldrich Station floras). Furthermore, white pine is now in the subalpine forest of the Sierra Nevada, well above the bigtree community, yet its Late Tertiary equivalent (*Pinus quinifolia*) formed an integral part of the forest in western Nevada, where it was in ecotone with oak woodland.

Temperature. Lowered winter temperature also appears to have had a critical role in the later developmental stages of the forest. Winter temperatures had been decreasing gradually during the Tertiary, and reached a low point in the Middle Pliocene. Following a slight rise in the Late Pliocene, temperatures dipped rapidly as the Ice Age set in.

A number of trees and shrubs of the

Sierra-Cascade Component that occurred with bigtree in the Late Tertiary have close relatives which live now within a few miles of the community, at warmer levels near the lower margin of the Sierra-Cascade forest. Segregation of these species of madrone, giant chinquipin, mock locust, box elder, ash, and nutmeg from the forest may have been a Quaternary event, in response to the development of colder winter climate in areas where bigtree survives.

The modern bigtree community apparently has become adapted to somewhat cooler and moister conditions than those under which the ancestral forest lived during the Late Tertiary. As the Sierra Nevada was elevated in the Pleistocene, precipitation increased on the west flank of the range where the forest lives. In addition, temperatures were lowered owing to increased elevation as well as to general climatic changes (Axelrod, 1957a). The shift of the forest to higher altitudes would compensate somewhat for the absence of summer rain because evaporation would be reduced at these cooler levels. Furthermore, increased precipitation in winter would provide a more constant source of ground water through the year, and especially during the hot dry summer season for this shallow-rooted giant. My observations suggest that high summer temperature and the extremely high evaporation rate in the Sierran forest below the bigtree groves militates against its establishment at lower altitudes. Even though large permanent streams and rivers now disperse its seeds well down the valleys, bigtree does not grow at the lower margin of the pine-fir forest except in unusually favorable micro-environments. In such sites shade from the hot summer afternoon sun is provided by high ridges, cool air drainage is regularly present into mid-morning, and the cold river water chills the immediate area to provide an oasis for its persistence. Thus it seems likely that if summer temperatures were more moderate today, bigtree might well survive naturally over a much wider area in the Sierran forest.

Temperature may also have had a role in developing differences between the northern and southern parts of the forest. The bigtree groves in the northern section of the range have some species, such as *Pseudotsuga taxifolia, Taxus brevifolia,* and *Vaccinium parvifolium,* which are largely relict there but occur commonly to northward in the Sierra-Cascade and Coast forests. In addition, the pine-fir forest in the northern Sierra Nevada has other species, including *Acer circinatum, Aralia californica, Berberis aquifolium, Castanopsis chrysophylla, Euonymus occidentalis, Lithocarpus densiflora,* and *Osmaronia cerasiformis,* which while known chiefly from local areas, are common in the Coast Ranges and northward. Many angiosperms of the Sierra-Cascade Component listed above (see Composition) also occur in the Sierra-Cascade and Coast forests in northwestern California and attest to their common origin. These modern communities have been differentiated quite recently, and largely in response to temperature, with the Coast Forest living under a more equable climate than the Sierra Cascade. The comparatively mild, wet winters at the north end of the Sierra Nevada may thus account for the presence there of the species that link both forests, but which have only a relict occurrence in the Sierra. The hot summers and accompanying high evaporation in the southern section of the range may well explain the absence there of these plants whose fossil relatives appear to have had a wider occurrence in the ancestral Late Tertiary community.

Madro-Tertiary species that formerly ranged to the margins of the forest are represented now by closely related plants in oak woodland country well below forest, or else occur to southward. They appear to have been eliminated from the forest and its margins during the Early Quaternary as temperatures were lowered, since their living relatives are in

areas of winter climate milder than that near or in the bigtree forest today. Thus we may infer that these plants comprise a group whose range of tolerance for winter cold was insufficient to permit their persistence near bigtree as colder climate developed in the Sierra Nevada during the Pleistocene, and as the bigtree community became adjusted to it.

Living derivative species of the Conifer Woodland Element (see Composition) no longer occur near the bigtree community, though some of them range to the drier, colder margins of the Sierra-Cascade forest in western Nevada and adjacent California today. These species of *Amelanchier, Cercocarpus, Juniperus,* and *Symphoricarpos* appear to represent plants whose range of tolerance for low winter temperature has recently increased, and they have thus become adapted to the cold-winter climate of the Great Basin and border areas of which the piñon-juniper community is so characteristic. Judged by their associates, the present species (ecotypes?) are quite different physiologically from those of the Late Tertiary which survived close to bigtree, though the fossils are indistinguishable from the living. Other species of this element, such as *Cercocarpus holmesii* (*paucidentatus*) and *Fraxinus millsiana* (*anomala*), were restricted farther east, to areas of cold winters and summer rain.

Summer drought. The Quaternary bigtree community differed materially from the Late Tertiary association: all the survivors were well adapted to summer drought. During the Quaternary there was a significant regrouping of these species. This notably involved the final rise to dominance of the conifers—yellow pine, white fir, incense cedar, sugar pine—that now typify the association together with bigtree. This relation parallels the history of the other conifer forests that now occur in the far West, for nowhere in the Tertiary are there forest communities like these living associations which are dominated solely by conifers. Fossil relatives of the present day dominants of the cedar-hemlock, Douglas fir, redwood, and yellow pine forests all had a more subordinate role in the ancestral communities, which included numerous species whose nearest relatives now survive in areas of ample summer rainfall. As the latter were being eliminated in the Pliocene, conifers increased in abundance, and the final disappearance of species of these eastern elements enabled the conifers to assume full dominance. It is evident that they were either preadapted to a climate of summer drought, or else there has been evolution of tolerance in this direction.

Glaciation. Glaciation apparently had an important role in finally shaping the distribution of the present bigtree groves. Some eighty years ago, John Muir noted that the major groves are isolated by the large canyons which were glaciated. He suggested that bigtree may have formed a more nearly continuous forest in the range prior to glaciation, and that its present discontinuous distribution was due to its elimination from the valleys down which the glaciers advanced (1876, p. 250):

> Finally, pursuing my investigations across the basins of the Kaweah and Tule, I discovered that the Sequoia belt attained its greatest development, just where, owing to the topographical peculiarities of the region, the ground had been most perfectly protected from the main ice-rivers, that continued to pour past from the summit fountains, long after the smaller local glaciers had been melted.
>
> Beginning at the south, the majestic, ancient glaciers are seen to have been shed off right and left down the valleys of Kern and King's Rivers, by the lofty protective spurs outspread embracingly above the warm Sequoia-filled basins of the Kaweah and Tule. Then next northward comes the wide Sequoia-less channel of the ancient San Joaquin and King's River *mer de glace.* Then the warm, protected spots of the Fresno and Mariposa groves. Then the Sequoia-less channel of the ancient Merced glacier. Next the warm, sheltered ground of the Merced and Tuolumne groves. Then the Sequoia-less channel of the grand ancient *mer de glace* of the Tuolumne and Stanislaus; and lastly the warm, old ground of the Calaveras groves.

Bigtrees probably have not been able to rëestablish themselves in the major glaciated canyons because of cold air drainage. They do not live in valleys where subzero temperatures are prolonged, but in areas where temperature falls only occasionally below zero, and then for but brief intervals. Furthermore, where they occur in valleys which were occupied by the glaciers, like the groves along the major forks of the Kings and Kaweah Rivers, they live chiefly in the tributary canyons and valleys well above the main gorges down which the glaciers moved, and down which subzero air regularly penetrates in winter today.

Summary

The Late Cenozoic history of the bigtree community has been analyzed in terms of the changing spatial and floristic relations of an ancestral forest of diverse origins. Most species in the modern community are of Arcto-Tertiary derivation, but Madro-Tertiary plants are associated with it, chiefly at lower altitudes. More numerous species of both geofloras were associated with the forest during Miocene and Pliocene times.

Occupying western Nevada during the Late Miocene, the ancestral bigtree forest migrated to the windward slopes of the Sierra Nevada as rainfall decreased during the Pliocene. During and following this migration, the selective influence of changing climate (disappearance of summer rain, increasing winter precipitation, greater ranges and extremes of temperature), acting on the varying ranges of tolerance of the species and their ecotypes, greatly modified the composition of the community. Species eliminated from it include (1) Arcto-Tertiary plants whose nearest relatives survive now (a) in the Sierra-Cascade forest at either higher or lower altitudes, (b) in the Coast Forest, (c) in the Rocky Mountain forest, and (d) in the hardwood deciduous forests of eastern Asia and eastern America, and (2) Madro-Tertiary plants whose modern counterparts occur chiefly (a) in woodland country at lower levels in the Sierra Nevada or to southward, (d) in the conifer woodland of the Great Basin and areas to the east, and (c) in southwestern North America.

Those that survived to form the living community were either preadapted to a summer-dry climate, or evolved ecologic races that tolerate these conditions. Conifers, which had gradually been increasing in numbers during the Pliocene when plants requiring summer rain were being eliminated, finally assumed dominance in the Quaternary.

Differences between the northern and southern parts of the bigtree community, and at its upper and lower margins, developed chiefly in the Quaternary in response to the temperature, as well as moisture, relations of the component species that were segregated in response to these factors.

Glaciation in the Sierra Nevada apparently disrupted a more nearly continuous bigtree forest to give rise to the present scattered groves that occur between the major glaciated canyons.[8]

Literature Cited

Axelrod, D. I. 1937. A Pliocene flora from the Mount Eden beds, southern California. Carnegie Inst. Wash. Pub., **476**: 125–183.

——. 1939. A Miocene flora from the western border of the Mohave Desert. Carnegie Inst. Wash. Pub., **516**: 1–129.

——. 1941. The concept of ecospecies in Tertiary plaeobotany Proc. Natl. Acad. Sci., **27**: 545–551.

——. 1940a. The Pliocene Esmeralda flora of west-central Nevada. Jour. Wash. Acad. Sci., **30**: 163–174.

——. 1940b. Late Tertiary floras of the Great Basin and border areas. Bull. Torrey Bot. Club, **67**: 477–487.

——. 1944a. The Oakdale flora (California). Carnegie Inst. Wash. Pub., **553**: 147–165.

——. 1944b. The Sonoma flora (California). Carnegie Inst. Wash. Pub., **553**: 147–165.

——. 1944c. The Alvord Creek flora (Oregon). Carnegie Inst. Wash. Pub., **553**: 225–262.

[8] This paper is an outgrowth of studies in paleobotany which have been generously supported by the National Science Foundation.

——. 1948. Climate and evolution in western North America during Middle Pliocene time. Evol., **2**: 127–144.

——. 1950a. Classification of the Madro-Tertiary flora. Carnegie Inst. Wash. Pub., **590**: 1–22.

——. 1950b. A Sonoma florule from Napa, California. Carnegie Inst. Wash. Pub., **590**: 23–71.

——. 1950c. The Piru Gorge flora of southern California. Carnegie Inst. Wash. Pub., **590**: 161–214.

——. 1950d. Evolution of desert vegetation in western North America. Carnegie Inst. Wash. Pub., **590**: 215–360.

——. 1956. Mio-Pliocene floras from west-central Nevada. Univ. Calif. Publ. Geol. Sci., **33**: 1–322.

——. 1957a. Late Tertiary floras and the Sierra Nevadan uplift. Bull. Geol. Soc. Amer., **68**: 19–46.

——. 1957b. Paleoclimate as a measure of isostasy. Amer. Jour. Sci., **255**: 690–696.

——. 1958a. Evolution of the Madro-Tertiary Geoflora. Bot. Rev., **24**: 433–509.

——. 1958b. The Pliocene Verdi flora of western Nevada. Univ. Calif. Publ. Geol. Sci., **34**: 91–160.

——. (MS). The Pliocene Chalk Hills flora of western Nevada. Univ. Calif. Publ. Geol. Sci.

Cain, S. A. 1944. Foundations of plant geography. Harper & Bros., New York. 556 pp.

Chaney, R. W. 1938. Paleoecological interpretations of Cenozoic plants in western North America. Bot. Rev., **4**: 371–396.

——. 1944. Introduction (to Pliocene floras of California and Oregon). Carnegie Inst. Wash Pub., **553**: 1–19.

——. 1948. The ancient forests of Oregon. Condon Lectures, Oregon State System of Higher Education. Eugene, Oregon. 56 pp.

——. 1959. Miocene forests of the Columbia Plateau. Carnegie Inst. Wash. Pub. **617**.

——, and H. H. Hu. 1940. A Miocene flora from Shantung Province, China. Carnegie Inst. Wash. Pub., **507**: 1–147.

Clements, F. E. 1916. Plant succession. Carnegie Inst. Wash. Pub., **242**: 1–512.

Condit, C. 1944a. The Remington Hill flora (California). Carnegie Inst. Wash. Pub., **553**: 21–55.

——. 1944b. The Table Mountain flora (California). Carnegie Inst. Wash. Pub., **553**: 56–90.

Good, R. D'O. 1931. A theory of plant geography. New Phytol., **30**: 149–171.

MacGinitie, H. D. 1953. Fossil plants of the Florissant beds, Colorado. Carnegie Inst. Wash. Pub., **599**: 1–188.

Mason, H. L. 1934. Pleistocene flora of the Tomales formation. Carnegie Inst. Wash. Pub., **415**: 81–179.

——. 1936. The principles of geographic distribution as applied to floral analysis. Madroño, **3**: 181–224.

——. 1947. Evolution of certain floristic associations in western North America. Ecol. Monogr., **17**: 201–210.

Muir, J. 1876. On the post-glacial history of *Sequoia gigantea*. Proc. Amer. Assoc. Adv. Sci., **25**: 242–253.

POLYPLOIDY IN GYMNOSPERMS

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Received April 9, 1958

The gymnosperms constitute a primitive group of seed plants which have a considerable evolutionary interest. A substantial amount of literature on the morphology of the group exists, but very little has been written about the various cytogenetic factors responsible for evolution within the group. Our knowledge of such factors is based solely on the studies of the present-day taxa which, indeed, offer but a mere apology for the glorious past of the group as a whole. In this communication the role of one such factor, polyploidy, has been evaluated. Polyploidy is found at three levels in gymnosperms: polyploid seedlings in the progeny of diploid species, isolated polyploid trees in otherwise strictly diploid species and lastly, polyploid species and genera. These will be discussed in turn.

POLYPLOID SEEDLINGS

These are stray seedlings that arise spontaneously in the progeny of diploid species and so far have been noted within five species whose details are given in table 1.

As is evident from the table these seedlings arise in very low percentages. Except in the case of *Welwitschia mirabilis* all the polyploid seedlings have been discovered in nurseries where they are conspicuous by their slow growth. Because of the aberrant growth of such seedlings, it appears that these were always discarded, until Kiellander (1950) first found that such aberrant seedlings were often aneuploid or polyploid. Nothing definite is known about their origin. It is likely that these seedlings arise either through a chance union of reduced and unreduced gametes or through chromosome doubling in embryonal initials during proembryo formation or early embryogeny. The former hypothesis would explain the origin of the triploid seedlings, but the more probable origin of the tetraploid seedlings is perhaps due to the chromosome doubling in embryonal initials. It is of interest to note that Illies (1953) discovered such seedlings in the progeny of the polyembryonic seeds of *Picea abies*.

In the opinion of the present writer these seedlings do not represent successful cases of polyploidy. They are comparable to the frequent reports of the origin of isolated polyploid seedlings within many of our crop plants. Furthermore, except for *Cryptomeria japonica* (Chiba, 1950; Zinnai and Chiba, 1951), all such seedlings are short, stumpy and very slow-growing. These characters do not equip them for competition in natural habitats where there are several competitors for any new niche opened for colonization. Under such conditions natural selection would favor fast-growing individuals. It is reasonable to assume that in nature such polyploid and aneuploid seedlings may be arising constantly in most of the diploid gymnosperms, but due to their defective growth such seedlings have no survival value whatsoever and are therefore constantly weeded out. The polyploid and aneuploid seedlings may be at best looked on only as "potentialities" of the diploid species. A parallel case has been reported by Jones (1954) in *Holcus*. He found only four races (4x, 5x, 6x, and 7x) occurring in nature, but seedlings raised in nurseries indicated that, in addition to these four races, there appeared several aneuploid types. The aneuploids were never found in nature, where they seem to be con-

TABLE 1. *Polyploid seedlings in* Gymnosperms

Species	2n-chromosome number of polyploid seedlings	Percentage	Author
Pinus densiflora (2n = 24)	48	0.08	Zinnai, 1952
P. radiata (2n = 24)	48	—	Rodger, 1953–54
Picea abies (2n = 24)	±28, 36, 48 28–30, 36	0.00216 —	Kiellander, 1950 Illies, 1953
Cryptomeria japonica (2n = 22)	33, 44	—	Chiba, 1950 Zinnai and Chiba, 1951
Welwitschia mirabilis (2n = 42)	84	2.127	Fernandes, 1936

stantly wiped out. Therefore, it may be concluded: 1) that aneuploid and polyploid seedlings in gymnosperms, and also in other plants appear in nurseries because in such habitats there is hardly any competition among the individuals, and 2) that the breeders encourage these seedlings since they now focus more attention on such aberrants.

POLYPLOID TREES

Two solitary cases of polyploid trees have been reported: one each in *Larix decidua* (Christiansen, 1950) and *Juniperus virginiana* (Stiff, 1951 and unpublished).

The investigations of Sax and Sax (1933) and of Christiansen (1950) have shown that the prevailing cytological situation in *Larix decidua* (syn. *L. europea*) is that it is a diploid (2n = 24). A single tetraploid tree (2n = 48) was found growing in an estate in Denmark and studied by Christiansen (1950). From its morphology and also from the cytological behavior the tree, as expected, was found to be an autotetraploid. A preponderance of quadrivalents in addition to such irregularities as bridges, laggards, micronuclei, polyads, pollen grains with irregular chromosome numbers and low fertility was observed. The trunk of this tree is thinner than that of the diploids. From a study of the growth rings, it is clear that the tree has had a poor start; then suddenly it got a fillip, and later the growth rate again became very low. No data are available regarding its sexual progeny, but it seems reasonably certain that the progeny may not be cytologically balanced because of the irregularities, and in particular because of the deviating chromosome numbers in the pollen grains.

Juniperus virginiana has been reported as diploid (2n = 22) by several workers (Sax and Sax, 1933; Mathews, 1939; Löve and Löve, 1948; Ross and Duncan, 1949; Mehra and Khoshoo, 1956a), and also by Stiff (1951). The latter found one triploid (2n= 33) plant (about 4 ft in height) in the Orland E. White Arboretum of the Blandy Experimental Farm. Furthermore, he noted that the triploid does not differ in any qualitative character from the diploid, but shows gigas characters, as evidenced by the larger dimensions of stomata and nuclear volume. In view of this, it is more or less clear that the triploid plant is autotriploid.

It is certain that both instances are solitary cases of autoploidy in otherwise strictly diploid species. Furthermore, it appears that what is dealt with here are those polyploid seedlings which have been able to thrive and in one case (*Larix decidua*) even grow to maturity.

The reason for their success lies in the fact that both of these plants happen to have occupied protected habitats (an estate or an experimental farm) where, as indicated earlier, selective forces are not against such individuals. These two cases are comparable to the several reports regarding the occurrence of sporadic autoploid individuals in experimental fields in many of our crop plants.

With all their inherent limitations, both the polyploid seedlings and polyploid trees undoubtedly merit a very careful study of their morphology, cytogenetics and particularly of their economic properties. More important is the possibility that they could in time be utilized for raising auto- or allotriploid strains which are useful at least in some angiosperm tree species (Müntzing, 1936b; Johnsson, 1945, 1950, 1953). In *Populus tremula* the triploids are more vigorous than the diploids and tetraploids (Johnsson, 1953), which is perhaps due to hybridity rather than polyploidy itself. In this connection it is of interest to note that the triploid *Larix* raised after interspecific hybridization by Syrach-Larsen and Westergaard (1938) is also fairly vigorous.

Polyploid Species and Genera

A species or a genus can be regarded as polyploid only if most (if not all) of its members are polyploid. In this sense, isolated polyploid seedlings and trees are only fortuitous cases. A perusal of the existing cytological literature on gymnosperms reveals that there are two types of gymnosperms that have been regarded as polyploid. In one class polyploidy is only of an apparent kind, being due to the increase in chromosome number as a result of causes not associated with the origin of polyploids. On the other hand, in the other class the taxa are genuinely polyploid. To the first category belong *Pseudolarix amabilis* (Sax and Sax, 1933) *Podocarpus* species (Flory, 1936; Mehra and Khoshoo, 1956b), and, perhaps, also *Welwitschia mirabilis* (Florin, 1932; Fernandes, 1936).

Pseudolarix possesses n = 22, which number is unique for Pinaceae, since most of its genera contain n = 12. Out of the 22 chromosomes in *Pseudolarix*, there are 20 terminal or subterminal and two nearly median chromosomes. By comparing this karyotype with the karyotype of the allied genera like *Pinus, Cedrus* and *Larix* (all n = 12 and with no terminal chromosomes) it is easy to imagine that the karyotype of *Pseudolarix* has arisen by the fragmentation of ten (out of 12) median-submedian chromosomes through the region of centromere. This would result in 20 terminal and two median-submedian chromosomes. The centromere of the terminal chromosome could become subterminal through an inversion involving the centromere. Such a suggestion was rejected by Sax and Sax (1933), in view of the well known postulate of Navashin (1932) about the centromere. Instead, Sax and Sax (1933) put forward the suggestion that *Pseudolarix* is a hypotetraploid (4x − 4) with 12 as the basic number. In this connection it is pertinent to note that the original postulate of Navashin (1932) has lost much of its rigidity, and data have accumulated which go a long way to show that fragmentation of median-submedian chromosomes into terminal chromosomes is possible. Such a mechanism has been inferred directly or by implication in several instances involving plant material by various workers (cf. Levan, 1932, 1935; Levan and Emsweller, 1938; Beal, 1939; Darlington, 1939, 1940; Rhoades, 1940; Garber, 1944; Chakravorty, 1948; Darlington and LaCour, 1950; Sundar Rao, 1950; Sears, 1952 a, b; Darlington, 1956; see these papers for further references). In view of this fact, the present writer feels that a more reasonable explanation of the origin of *Pseudolarix* is in the fragmentation of median chromosomes into terminal chromosomes, and not in autoploidy followed by a loss of four chromosomes. The

mechanism of fragmentation is probably misdivision (Darlington, 1939, 1940; Darlington and LaCour, 1950). The material basis for the process of misdivision is to be looked for in the compound nature of the centromere so clearly brought out by Lima-de-Faria (1949, 1956) and, particularly, by Tjio and Levan (1950).

Similarly, *Podocarpus* species with n = 19 may have been derived by fragmentation of median-submedian chromosomes of the species with lower numbers (cf. Flory, 1936; Mehra and Khoshoo, 1956b). However, a future cytogenetic investigation of the family Podocarpaceae may reveal the true situation. At the moment, the second suggestion of Flory (1936) that *Podocarpus* species with n = 19 are triploids from n = 13 cannot be regarded as valid. The simple reasoning here is that to date we do not know of any sexual triploid species ordinarily breeding true for its chromosome number. However, in view of the great antiquity of both Pinaceae and Podocarpaceae, one cannot be very sure of the manner in which chromosome number has increased in *Pseudolarix* and *Podocarpus* species, since there is no certainty regarding the probable ancestors of these genera. At any rate, no living genus can be regarded as the ancestor of these genera. The present evidence clearly reveals that the karyotypes of both *Pseudolarix* and *Podocarpus* species (n = 19) are neither exact multiples nor show any qualitative resemblance to their low-numbered relatives, and are therefore not polyploids of the latter.

Further support for the suggestion that *Pseudolarix* and *Podocarpus* species are not polyploids can be derived from the consideration of a comparable case in cycads. Sax and Beal (1934) have found that in the genus *Microcycas* (n = 13) out of 13 chromosomes, 11 are terminal, one subterminal and one has a median centromere. There is, however, a complete unanimity of opinion that *Microcycas* is not a polyploid of cycads with low numbers and a symmetrical karyotype. We are therefore hardly justified in regarding *Pseudolarix* or *Podocarpus* species as polyploid, because karyotypically both these cases are comparable to *Microcycas*.

Welwitschia has 2n = 42 (Florin, 1932; Fernandes, 1936), and is often interpreted to be a hexaploid because of the basic number of the genus *Ephedra* (x = 7). The analysis of the karyotype of *W. mirabilis* given by Fernandes (1936) clearly indicates that the 42 chromosomes of the somatic complement can only be classed in two basic sets of 21 chromosomes each. There is, however, no indication that the basic karyotype is composed of seven chromosomes, since in that case it should have been represented six times. At this stage it is pertinent to note that except *Podocarpus* many of the gymnosperm genera worked out to date show in general a stability of the basikaryotype not only within their species (cf. Sax and Sax, 1933; Sax and Beal, 1934; Flory, 1936; Mehra, 1946; Mehra and Khoshoo, 1956a, b) but also in their polyploids (Mehra, 1946a; Christiansen, 1950; Knaben, 1953; Hunziker, 1955). In strong contrast to *Welwitschia,* in the genus *Ephedra,* Mehra, (1946a) and Hunziker (1955) have found that in the tetraploid species the basikaryotype is represented four times. Therefore there is a complete lack of a basikaryotype of seven chromosomes in *Welwitschia,* unless we believe that in this genus alone there were radical alterations in the karyotype following polyploidy. The investigation of Fernandes (1936) has further shown that in *Welwitschia* the number of nucleoli is only two as is expected of a diploid. Furthermore, some of the chromosomes are telocentric. All these facts, when taken together, strongly point that the situation in *Welwitschia* should not be interpreted on the basis of *Ephedra.* However, a more important argument in support of the above conclusion is that the two genera (*Ephedra* and *Welwitschia*) are quite different morphologically (using the word in its widest sense) and

phytogeographically (cf. Arnold, 1948; Eames, 1952; Takhtajan, 1953; Florin, 1955; see these for further references). In view of these facts it is not as yet certain whether the resemblances between the two genera are of any real significance. They may only indicate parallel evolution in two otherwise more or less unrelated stocks. The chromosome number of *Welwitschia* is fairly high ($2n = 42$), and surely the higher the number of chromosomes, the more ways there are in which it could be compounded (cf. Stebbins, 1950). Thus, one cannot be very sure as to how the higher number has arisen, especially when the genus is not only monotypic but even the order Welwitschiales is monogeneric. With the present state of knowledge of the genus *Welwitschia*, the question of its ploidy level should be kept open.

Gnetum ($n = 22$) is yet another gymnosperm whose number is apparently indicative of polyploidy, especially because Fagerlind (1941) observed 11 smaller and 11 bigger bivalents in pollen mother cells of *G. gnemon*. It is interesting to note that according to Cook (1939) there are some resemblances in embryogeny between *Gnetum* ($n = 22$) and *Juniperus* ($n = 11$, Cupressaceae). However, such resemblance may be far-fetched, and the question of polyploid nature of *Gnetum* should wait till more species of the genus have been worked out.

There are only 11 gymnosperm species which can be regarded definitely polyploid in constitution. These are: *Sequoia sempervirens* (Hirayoshi and Nakamura, 1943; Yasui, 1946; Stebbins, 1948), *Juniperus chinensis pfitzeriana* (Sax and Sax, 1933), *J. squamata meyeri* (Jensen and Levan, 1941); the remaining eight cases occur in the genus *Ephedra* (Florin, 1932; Resende, 1937; Mehra, 1946a; Hunziker, 1953, 1955; Krapovickas, 1954). We may now proceed to comment critically on each of these.

The cytology of *Sequoia sempervirens* was correctly worked out for the first time by Hirayoshi and Nakamura (1943) and by Yasui (1946). Stebbins (1948), unaware of these publications also studied the species having $n = 33$, a number now confirmed by M. L. Stiff (personal communication) who counted 66 chromosomes in somatic tissue. The numbers $n = 33$ and $2n = 66$ indicate that the species is a hexaploid, when compared with $n = 11$ found in all the genera of the family Taxodiaceae (cf. Mehra and Khoshoo, 1956a, table 2). That this species has in all probability remained a hexaploid since Tertiary (Pliocene) is apparent from the work of Miki and Hikita (1951). These writers have correlated the epidermal and guard-cell dimensions with chromosome number in the present day *Sequoia* (6x) and *Metasequoia* (2x). On the basis of this correlation they have deduced that the probable somatic chromosome number in fossil Sequoias of Japan was 66 and that of fossil *Metasequoia japonica* was 22. The observations of Hirayoshi and Nakamura (1943) and of Stebbins (1948) indicate that there are hexa-, quadri-, bi- and univalents at meiosis in *Sequoia*. Stebbins (1948) is of the opinion that it is a case of auto-allohexaploidy which has arisen from at least two if not three parental species. He made a thorough comparative character-analysis of the species in relation to other Taxodiaceous genera. He rightly rejected the idea that *Sequoiadendron giganteum* is one of the parents because of the fundamental differences between it and *Sequoia*. Yasui (1946) is also of the same opinion. On the other hand Doyle (1945) believes that *Sequoiadendron giganteum* (*Sequoia gigantea*) is the type from which *Sequoia sempervirens* has been derived. Apart from the morphological evidence given by Stebbins (1948), it may be pointed out that, if Doyle's interpretation is correct, then the autotetraploid of *Sequoiadendron* raised by Jensen and Levan (1941) should have shown rather a closer resemblance to *Sequoia sempervirens* (6x). Actually that is not the case. Instead, Stebbins (1948) has convincingly come to the con-

clusion that *Metasequoia glyptostroboides* is likely to be one of the parents of hexaploid *Sequoia*.

The multivalent associations indicate that the sexual progeny of *Sequoia* would not be balanced cytologically. In this connection it is of interest to note that Doyle (1945) has observed a considerable gametophytic and embryological sterility in this species. Furthermore, Buchholz and Kaieser (1940) and Doyle (1945) have also noted a good deal of seed abortion. In view of the observations a question arises as to how *Sequoia* has been able to reproduce with fidelity from times immemorial. Available data shows that in this species there exists a capacity for vegetative reproduction (cf. Bailey, 1933; Buchholz, 1939a, b,; Dallimore and Jackson, 1948; Platt, 1953). It is a matter of common observation that this species not only has suckers from stumps which often produce "merchantable lumber" (Bailey, 1933) but even the burls sprout (Platt, 1953). This capacity for vegetative reproduction may explain to some extent the lack of diploidization in *Sequoia*. However, according to G. L. Stebbins (personal communication) there is no doubt that sexual reproduction in *Sequoia* is rare in the mature forests of the species, though he has several times observed, "literally thousands of seedlings of the species in the raw, gravelly soil of stream margins in the redwood belt, which appear in great numbers after landslides, where they actually occupy pioneer stages in ecological succession." He further remarks that, "although a considerable proportion of the seeds produced by *Sequoia* may be inviable, the actual number of seeds produced each year by a single large tree is so enormous that there are always plenty available to colonize every suitable ecological niche which occurs in the vicinity of old trees. Under these conditions, selection pressure for increased fertility would be very low, and this explains the persistence for millennia of meiosis with multivalents." Furthermore, because of prolific seed formation, there are greater chances of production of individuals containing parental genomes in full. Those that are unbalanced may be eliminated by selection during embryo development or during germination or, even later, in the seedling stage. In view of abundant seed formation such embryonal selection can occur without any harmful effect on the reproductive capacity of the species.

In conclusion, *Sequoia* is the solitary gymnosperm species which is hexaploid. It is unique, since it maintains itself in spite of the persistence of multivalents (Stebbins, 1948) and sterility (Buchholz and Kaeiser, 1940; Doyle, 1945). This is possible because of its capacity for vegetative reproduction, prolific seed production, and subsequent selection of genetically balanced individuals in new and unstable habitats. It may also be suggested that the wide geological distribution of this species throughout northern hemisphere may be in part due to its capacity for both sexual and vegetative reproduction.

The second case of polyploidy is *Juniperus chinesis pfitzeriana*, which possesses 22 bivalents and has 6% of pollen sterility (Sax and Sax, 1933). These writers are of the opinion that the species is an autotetraploid in which diploidization has taken place. It is quite possible that this line of argument may be the correct one. However, one point needs emphasis. The species has rather long chromosomes and yet shows normal meiosis. It has all the cytological characteristics of a perfect allotetraploid. May it not be that this species arose after an intercenospecific cross? Perhaps the only objection to such a suggestion is that, so far, all the *Juniperus* hybrids discovered are fertile and therefore exhibit intra- or interecospecific relationship.

The third polyploid in coniferales is *Juniperus squamata meyeri* reported by Jensen and Levan (1941). They have investigated only the root-tips of the species and therefore it is not possible to

comment on the nature of the polyploidy (personal communication from A. Levan).

Out of the eight polyploid species in *Ephedra,* five (*E. altissima, E. intermedia, E. likiagensis, E. saxatilis* and *E. sinica*) have been analyzed by Mehra (1946a). He has shown that all these species are allotetraploid as indicated by karyotypic analysis. Though every tetraploid species contains a basikaryotype represented four times, yet, due to the differences in number and nature of nucleolar organizers, the entire complement can be divided into only two sets of 14 chromosomes each. Furthermore, meiotic analysis of a few tetraploid species has revealed that there is bivalent pairing, although some cytological disturbances at various stages and also some degree of sterility occur (Mehra, 1946b). *E. distachya* (Florin, 1932; Resende, 1937) is a tetraploid but has not been so far analyzed. *E. americana* (sensu lato, cf. Index Kewensis) contains not only *E. americana* proper, but also *E. andina* and *E. rupestris.* Out of these, *E. americana* (sensu stricto) and *E. rupestris* are diploids, while *E. andina* has been reported as diploid, tetraploid and hypertetraploid with $2n = 30$ chromosomes (Hunziker, 1953, 1955). Only the tetraploid form has been on karyotypic grounds deduced to be an allotetraploid (Hunziker, 1955). *E. breana,* though reported as diploid by Hunziker (1953, 1955), has, however, been found to be a tetraploid by Krapovickas (1954). It therefore seems that *E. americana* (sensu lato) and *E. breana* contain intraspecific chromosomal races, and need a closer cytotaxonomic study.

The following is the analysis of the natural polyploids reported so far in gymnosperms:

(i) Auto-allohexaploidy:	*Sequoia sempervirens*
(ii) Allotetraploidy:	***Juniperus chinensis pfitzeriana, Ephedra altissima, intermedia, likiagensis, saxatilis, sinica*** and the tetraploid form of *E. americana* (= *E. andina*)
(iii) Undetermined:	*Juniperus squamata meyeri, Ephedra breana* (tetraploid form) and *E. distachya*
(iv) Apparently intraspecific polyploidy:	*Ephedra americana* (sensu lato) and *E. breana*
(v) Further cytogenetic studies are desired on:	All *Juniperus* and *Ephedra* polyploids

Causes of Rarity of Polyploids

The foregoing survey has revealed that so far there have been only eleven authentic cases of polyploidy distributed in various orders reported. The data on the number of cytologically determined species within the various orders from an unpublished manuscript of the writer are summarized in table 2, which reveals that, in strong contrast to other plant phyla, the gymnosperms contain a very low percentage of polyploids. The question as to why polyploidy is rare in gymnosperms then arises. Various explanations have been suggested from time to time.

The first hypothesis was advanced by Sax (1932). She found a preponderance of interstitial chiasmata at meiosis. This fact was later confirmed by Sax and Sax (1933) and by Andersson (1947). According to Sax, quadrivalents with such chiasmata are likely to cause irregularities in meiosis, which would in turn cause sterility. Polyploids then would have a "small chance of survival." Heilborn (1934) has criticized this hypothesis since

TABLE 2. *Polyploids in* Gymnosperms

Order	Total number of species	Number of species worked out	Number of polyploids	Percentage of polyploids
Cycadales	ca. 85	23	—	—
Ginkgoales	1	1	—	—
Coniferales	ca. 483	195	3	ca. 1.5
Ephedrales	ca. 35	18	8	44.4
Welwitschiales	1	1	—	—
Gnetales	ca. 30	2	—	—
Total	ca. 635	240	11	ca. 4.6

interstitial chiasmata are not universally present in gymnosperms. Dark (1932) found many of the chiasmata to be terminal, and similar observations have been made by Khoshoo (1957) on normal plants of *Cephalotaxus*.

Multivalents are a constant feature of autoploids (like *Larix decidua,* cf. Christiansen, 1950) or auto-alloploids (*Sequoia sempervirens,* cf. Hirayoshi and Nakamura, 1943; Stebbins, 1948) in gymnosperms. In these cases multivalents contribute to the sterility. On the other hand, if the polyploid is alloploid (*Juniperus chinensis pfitzeriana,* Sax and Sax, 1933; *Ephedra* species, Mehra, 1946b), there is only bivalent pairing and sterility is absent or low. Therefore, while Sax's hypothesis may explain the rarity of auto- or auto-alloploids in gymnosperms, it cannot explain the rarity of alloploids, which is more a question of cytogenetic differentiation of species.

Another hypothesis was put forward by Müntzing (1933, 1936a), who is of the opinion that double fertilization is the factor involved in the preservation of polyploidy in angiosperms. He states that the cytological ratio between the ovular tissue, endosperm and embryo normally is 2:3:2. This ratio is seriously disturbed if a diploid and its polyploid cross. In support of his concept, he noted that there is rarity of polyploidy in a group without double fertilization. Apparently this hypothesis works very well with the gymnosperms, since this group is strictly cross-pollinated and has no double fertilization. Therefore, a polyploid could easily cross with its diploid progenitor or progenitors. Generation after generation a polyploid race would break up and lose its identity, unless it developed some kind of barriers. However, this concept cannot explain the preponderance of polyploidy in Ephedrales where there is no double fertilization either. Furthermore, it cannot explain the occurrence of polyploidy in other plant phyla lacking both endosperm and double fertilization, viz. Pteridophyta (cf. Manton, 1950; Manton and Sledge, 1954), mosses (cf. Steere, 1954; Steere, Anderson and Bryan, 1954) and liverworts (cf. Tatuno, 1949; see also the list by Delay, 1953). Some of these groups have a very high frequency and grade of polyploidy.

Sax and Sax (1933) rightly believe that Müntzing's hypothesis could instead very well explain the occurrence of fewer genera and species in gymnosperms, because there would be free hybridization, so that, due to free recombination, distinctions between groups would break down. No doubt the cause of fewer genera and species may also lie in the extinction of taxa.

Darlington (1937) put forth the view that the relationship between the number and size of chromosomes and cell size acts as a limiting factor for polyploidy. He states that with polyploidy the chromosome number multiplies but the area of the metaphase plate does not increase proportionately. Evidently the ratio be-

tween the two is not maintained in polyploids. As a result, the chromosomes cannot arrange themselves properly during cell division, because of the small cell plate. Therefore, chromosome number and size would be limited by the smallest cell in the life history of the plant. In plants with secondary growth these are the cambial cells. In support of his concept, Darlington (1937) states that polyploidy is rather rare in genera like *Lilium* and *Fritillaria,* which possess the largest chromosomes and have no secondary growth. He considers the gymnosperms to be at the "upper limit," since these possess fairly large chromosomes and have secondary growth.

It is possible that in gymnosperms this ratio has reached an equilibrium. Any change (in the optimum chromosome number and cell size) by polyploidy proves deleterious to growth and development of the species. Indirect support for this contention comes from the observations on the polyploid seedlings occurring spontaneously and on those produced artificially by colchicine. In general, in both cases the growth is retarded and the seedlings are short and stumpy. The reason, perhaps, lies in the disturbance in size relationship of nucleus and cell.

Stebbins (1938, 1950) has substantiated the above concept and has pointed out that fiber cells are formed in angiosperms. These, being very small, must be formed from similarly very small cambial cells. In gymnosperms, in general, fibers are not present and the chromosome size is larger in comparison to most woody angiosperms.

It may then be said that gymnosperms are a group with secondary growth, without fibers and with long chromosomes. Therefore, the concept, substantiated by Stebbins, can explain the presence of long chromosomes in the group but cannot explain the rarity of polyploidy in general. It cannot explain the occurrence of the hexaploid *Sequoia sempervirens* (which is one of the gigantic trees of the world), of other successful cases of polyploidy in conifers and the preponderance of polyploidy in Ephedrales. It may be remembered that all these taxa have large chromosomes and secondary growth.

Yet another hypothesis could be developed, taking into consideration the cytogenetic relationship of the diploid species of gymnosperms.

In an unpublished manuscript, the writer has concluded that, in general, the gymnosperm genera (except notably *Podocarpus*) are homoploid, and speciation is a matter of gene mutation and/or repatterning of chromosomes. Furthermore, due to the lack of double fertilization and the presence of wind pollination, hybridization in gymnosperms is to be expected to be very common. This naturally explains the numerous reports on the natural and artificial intra- and interspecific hybridization within several gymnosperm genera: *Araucaria, Taxus, Pinus, Picea, Larix, Abies, Tsuga, Cupressus, Chamaecyparis,* and *Juniperus* (cf. Forestry Section, Plant Breeding Abstracts). Besides these, there are also intergeneric hybrids on record: *Ceratozamia* × *Zamia* (Chamberlain, 1926), four hybrids involving *Tsuga, Picea* and *Keteleeria* (Campo-Duplan and Gaussen, 1949) and *Cupressus* × *Chamaecyparis* (Osborn, 1940).

Many of the above hybrids (intra- and interspecific, and inter-generic) are fairly fertile. So far, the writer has not found a report of a case of a completely sterile interspecific or intergeneric hybrid in gymnosperms in which sterility is chromosomal due to a lack of homology of chromosomes. On the other hand, generally bivalent pairing has been found in hybrids wherever such studies have been made (Sax, 1932; Sax and Sax, 1933; Hirayoshi, Nakamura and Kano, 1943; Ross and Duncan, 1949). In many cases, because of the homology of the chromosomes, there is not only fertility but also regular gene flow. These facts will be considered in detail in a subsequent publication; for the present discussion the

only important point is that, wherever hybridization is successful, the relationship between the two parents is below or at the ecospecific level, no matter whether the taxa involved are full fledged genera in the morphological sense. Therefore, hybridization is likely to result in "homogamic hybrid complexes" (cf. Grant, 1953). Keeping in mind the important postulate of Stebbins (1947, 1950) that polyploidy is almost always associated with hybridization, it appears certain that polyploids ensuing from "homogamic hybrid complexes" would be autoploid or, at best, segmentally alloploid. At any rate, it is more or less certain that the polyploids would have many autoploid characteristics. Naturally, such polyploids would possess multivalents, particularly because in gymnosperms chromosomes are fairly long. The validity of this statement is borne out by the cytological studies on true autoploids (*Larix decidua,* Christiansen, 1950) and also on those in which hybridization is involved (*Sequoia,* Stebbins, 1948; triploid *Larix,* Knaben, 1953). In all these cases there are multivalents and sterility, which in all probability is the result of irregular disjunction of multivalents, univalents, although it also may be due to physiological causes (cf. Stebbins, 1947). Such polyploids would neither breed true morphologically nor cytologically. Almost all gymnosperms reproduce predominantly by sexual means and because of this, it is doubtful if the "raw" gymnosperm polyploids with all their inherent limitations would be able to pass the "bottleneck" of initial sterility before they could establish themselves as fertile and true-breeding lines. The case of autotetraploid maize (Gilles and Randolph, 1951) is apparently an encouraging example, since, being a sexual annual, it showed progressive bivalent formation within ten years. However, the question remains whether autotetraploid maize can have survival value in nature and it is only then that a reduction of quadrivalents may be expected to take place. It may be pointed out that the situation in experimental fields is far too different from the one in nature. It is also pertinent to mention here that autoploids are not at all good competitors. This conclusion is borne out by the work of Sakai and Suzuki (1955a,b), and of the present writer (unpublished data on the *Sisymbrium irio* complex). According to all these workers, genomic alloploids are much superior in competitive ability to auto- or autoalloploids.

This explanation apparently does not hold for *Sequoia,* which is an auto-allohexaploid and possesses multivalents. As already indicated, the persistence of multivalents can be partly explained by the capacity of *Sequoia* for vegetative reproduction and by its exceptionally high seed production. Because of the latter, there is a greater probability not only for production of genetically balanced individuals but also of low selection pressure for increased fertility. It is because of such a combination of factors that *Sequoia* still retains autoploid characters. In the light of these statements *Sequoia* therefore does not contradict the above hypothesis.

In sexual pteridophytes and angiosperms almost all successful and vigorous polyploids have alloploid characteristics. Their origin is directly possible from intercenospecific crosses with chromosomal sterility, a situation singularly lacking in gymnosperms. Furthermore, in gymnosperms the origin of alloploids from auto- or segmental alloploids is problematic, perhaps, because of sterility and low competitive ability. The very fact that polyploids have not established themselves in gymnosperms (even though polyploid seedlings arise constantly) is a clear proof that alloploids cannot arise from auto- or segmental alloploids in this group. The few cases of alloploids occurring in gymnosperms may very well have arisen after rare intercenospecific crossing.

In support of the above contention, the examples of genera like *Aquilegia, Ceano-*

thus Quercus, etc., which are well known for ecospecific differentiation of species (cf. Stebbins, 1950), could be cited. In all these genera there are either no polyploids or their percentage is very low (cf. Darlington and Wylie, 1955).

The low frequency of polyploidy is also explained by the fact that agamospermy is, perhaps, totally lacking in the group (at any rate, so far, there is no authentic case reported in literature). Furthermore, the incidence of vegetative reproduction is also very low. It is not necessary to mention the fact that raw polyploids have a better chance of survival if potentialities for agamospermy and/or vegetative reproduction are present in diploid species. It may be pointed out here that the observation of Land (1913) regarding the possible vegetative reproduction in *Ephedra* are significant, in view of the high incidence (44.4%) of polyploidy in this genus.

The gymnosperms (especially *Ginkgo* and Coniferales) resemble woody angiosperms in their habit, and, in general, lack cases of polyploidy for reasons enumerated above (cf. also Stebbins, 1938, 1950). Both groups show stability not only in their habit and chromosomes but also in that both occupy relatively stable mesophytic habitats where they form great forest belts. It is true that the basic numbers of several woody angiosperms may be themselves of a polyploid origin (Stebbins, 1947, 1950), and this could very well act as an additional factor accounting for a general lack of polyploids. However, there is so far no evidence for the polyploid origin of the basic numbers of the majority of gymnosperms. Furthermore, the cytological stability of gymnosperms and also of woody angiosperms is not a modern attribute but appears to have been handed down from geological times, Paleozoic in the former and Cretaceous in the latter. Keeping these points in mind, it should be emphasized that the lack of polyploids, at least in conifers, is not due to any inherent features of this group alone, but that conifers are woody plants and like other woody plants (whether gymnosperms or angiosperms) lack polyploids for reasons discussed earlier. Furthermore, Stebbins (1950) has already made the important generalization that polyploids occupy geologically newer habitats, and, it appears, that the stable and constantly favorable habitats in which woody-plants grow, do not offer such opportunities in abundance. As a result, in general, polyploid races in trees are unlikely to establish themselves.

In brief, the rarity of polyploidy in gymnosperms is chiefly due to the woody habit itself and to ecospecific differentiation of species. Polyploids which might arise would have autoploid characteristics. Such polyploids are usually sterile and have low competitive ability. The net result is that these polyploids lack survival value, especially because other supporting factors such as vegetative reproduction and agamospermy are lacking in gymnosperms. Before the validity of these statements is accepted, studies along the following lines are needed. Artificial polyploids from interspecific and intergeneric crosses should be raised and studied cytogenetically. Search for completely sterile hybrids needs to be intensified. Polyploids from such hybrids should also be studied. Furthermore, competitive ability of tetraploids should be tested against their diploid parents. Incidentally, some of these studies would give very useful information about the extent and nature of cytogenetic differentiation of species and genera.

Conclusions

The foregoing survey has revealed that polyploids are rare in gymnosperms. The genus *Ephedra* represents the only group in which this type of evolutionary change is common. Primarily, the causes of rarity of polyploidy in gymnosperms are to be looked for in the woody habit and ecospecific differentiation of taxa (between which hybridization is possible), even though two genera may be involved.

Perhaps, in *Ephedra* the diploid species are strongly differentiated cytogenetically, and hybridization is still possible between them. It appears that in most of the other gymnosperms hybridization is not possible with increased morphological and cytogenetical differentiation of species.

As a whole, gymnosperms constitute a group in which hybridization is quite common, frequency of polyploids is low and apomixis is almost absent. Only one "closed system"—*Sequoia sempervirens* (6x)—is found in the group. In strong contrast, there are several cases of closed systems in pteridophytes and angiosperms, notably in the former (cf. Manton, 1950; Stebbins, 1950; Abraham and Ninan, 1954; Verma, 1956). The evolutionary future of all such closed systems is very limited. One could therefore reasonably expect more progressive evolution in gymnosperms, since the taxa are predominantly at diploid level. However, such evolution is not possible at present because the group is an antique one and therefore several taxa (notably cycads and *Ginkgo*) have been subjected to selection from times immemorial. Thousands of mutations may have occurred ever since their origin, so that their genotypes are now depleted and senescent even though they have stayed on the diploid level.

In pteridophytes and angiosperms hybridization, polyploidy and apomixis have been so rampant as to have caused a great amount of reticulation in the phylogeny of taxa, and to date there is no agreed classification of these groups (Stebbins, 1947, 1950). It may be remarked that ever since their origin, the incidence of hybridization, polyploidy and apomixis seems to be so common that in future it would be very difficult to obtain a truly phylogenetic classification of these groups. On the other hand, in gymnosperms only the incidence of hybridization is high. The genera and species are fewer than in pteridophytes and angiosperms. The problems of classification are relatively simple. This is partly due to the heavy extinction that has taken toll of the group in the past, thus creating discontinuities. Therefore, taxa that were once morphologically connected and genetically related became well separated when the "links" perished. This is why genera in gymnosperms are generally well recognized and clear cut. The only other difficulty in erecting a truly phylogenetic classification lies in parallel mutations and convergent evolution. These factors might connect taxa that were otherwise unrelated.

Summary

The role of polyploidy in gymnosperms has been evaluated. Polyploidy is found in the progeny of diploid species, in stray trees of otherwise strictly diploid species, and, finally, entire species or genera may be of polyploid constitution.

So far, polyploid seedlings have been discovered in very low percentages in the progeny of only five species (table 1). Such seedlings are only "potentialities" of the diploid species and, because of their short, stumpy habit and slow growth, cannot establish themselves in nature where fast growing individuals would be at a selective advantage.

Only two polyploid trees, one each in *Larix decidua* (4x) and *Juniperus virginiana* (3x), have been discovered so far. Both of these are autoploid in nature and appear to have arisen from polyploid seedlings which happen to have occupied protected habitats and were thus able to thrive.

Two types of gymnosperm species and genera have been regarded as polyploid. In one group, the increase in chromosome number is not due to causes associated with the origin of polyploids, i.e., the polyploid condition is only apparent and not real. *Pseudolarix amabilis* ($n = 22$), *Podocarpus* species with $n = 19$ and *Welwitschia mirabilis* ($2n = 42$) belong to this group.

The second group contains eleven cases of true polyploids. Three of these (*Sequoia sempervirens,* and two *Juniperus*

species) belong to Coniferales, while the remaining eight are found in Ephedrales. Only one (*Sequoia*) is auto-allohexaploid, seven (*Juniperus chinensis pfitzeriana* and six *Ephedra* species) are allotetraploids, while the remaining three species (*Juniperus squamata meyeri* and two species of *Ephedra*) are so far undetermined. Taking all gymnosperms together the frequency of polyploidy is only 4.6%.

The various hypotheses advanced to explain the rarity of polyploidy in gymnosperms have been reviewed. It appears that the chief causes of such rarity are the stability in habit and habitat and ecospecific differentiation of all the taxa between which hybridization takes place; even when the two taxa may be two genera in morphological sense. The resulting polyploids from such hybrids are expected to possess autoploid characteristics. Due to multivalents, other meiotic irregularities and physiological causes, a good deal of sterility is expected. Associated with this is the low competitive ability found in autoploids in general. All these facts, coupled with the total lack of apomixis in gymnosperms, would make the success of polyploids highly unlikely. In gymnosperms such polyploids do not seem to evolve into perfect alloploids.

The problems of classification are relatively simple in gymnosperms because of the lack of reticulate phylogeny which is so characteristic of pteridophytes and angiosperms, because of rampant hybridization, polyploidy and apomixis in the latter two groups. The only difficulties in erecting a sound classification of gymnosperms lie in the discontinuities caused by extinction of taxa. Thus, related taxa appear to be distinct and unrelated, and because of parallel mutations, unrelated taxa appear to be related.

Acknowledgments

I owe a deep debt of gratitude to Prof. P. N. Mehra for inspiring me to take up cytology of gymnosperms and for his keen interest, to Prof. G. L. Stebbins, Jr. (Davis) for his valuable criticism of the manuscript and inestimable help, and to Dr. M. L. Stiff (Lafayette) for kindly allowing me to quote his unpublished observations. To Profs. P. Maheshwari (Delhi), O. E. White, W. S. Flory (Virginia) and Dr. Z. M. Illies (Schmalenbeck) I am grateful for their help with the literature.

Postscript

After the above paper was submitted for publication, a valuable work appeared on the "Chromosomal Evolution in the Podocarpaceae" by J. B. Hair and E. J. Beuzenberg (Nature 1958, **181**: 1584–1586). Fifty-two species have been studied which cover all the seven genera of the family. The haploid chromosome number ranges from 9 to 19. There is a "regular numerical relationship" between the number of metacentric and subtelocentric chromosomes. The wide range in the chromosome number is the result of "fragmentation" of metacentrics and/or "fusion" of subtelocentrics. Furthermore, as usual, true polyploidy is conspicuous by its absence. The results of this investigation have been incorporated in table 2 of the present paper.

Literature Cited

(References marked with asterisks have not been seen in the original)

Abraham, A., and Ninan, C. A. 1954. The chromosomes of *Ophioglossum reticulatum* L. Curr. Sci., **23**: 213–214.

Andersson, Enar. 1947. A case of asyndesis in *Picea abies*. Hereditas, **33**: 301–347.

Arnold, C. A. 1948. Classification of Gymnosperms from the view point of paleobotany. Bot. Gaz., **110**: 2–12.

Bailey, L. H. 1933. The cultivated conifers in North America. New York.

Beal, J. M. 1939. Cytological studies in relation to the classification of the genus *Calochortus*. Bot. Gaz., **100**: 528–547.

Buchholz, J. T. 1939a. The embryogeny of *Sequoia sempervirens* with a comparison of Sequoias. Amer. Jour. Bot., **26**: 248–257.

——. 1939b. Generic segregation of the Sequoias. Amer. Jour. Bot., **26**: 535–538.

——, and Kaeiser, M. 1940. A statistical study of two variables in the *Sequoia*—pollen grain size and cotyledon number. Amer. Nat., **74**: 279–283.

Campo-Duplan, Van M., et Gaussen, H. 1949. Sur quatre hybrides de genres chez les Abietacees. Bull. Soc. Hist. Nat. Toulouse, **84**: 95–109. Vide Pl. Breed. Abstr. 1953, **23**: No. 695.

CHAKRAVORTY, A. K. 1948. Theory of fragmentation of chromosomes and evolution of species. Science and Cult., **13**: 309–312.

CHAMBERLAIN, C. J. 1926. Hybrids in cycads. Bot. Gaz., **81**: 401–418.

*CHIBA, S. 1950. Triploids and tetraploids of Sugi (*Cryptomeria japonica* D. Don.) selected in the forest nursery. Bull. Govt. For. Expt. Sta. No. 49, 99–108.

CHRISTIANSEN, H. 1950. A tetraploid *Larix decidua Miller*. Det. Kgl. Danske Vidensk. Selsk, **19**: 1–9.

COOK, P. 1939. A new type of embryogeny in the conifers. Amer. Jour. Bot., **26**: 138–143.

DALLIMORE, W., AND JACKSON, A. B. 1948. A handbook of Coniferae. London.

DARLINGTON, C. D. 1937. Recent advances in cytology. London.

——. 1939. Misdivision and the genetics of centromere. Jour. Genet., **37**: 341–364.

——. 1940. The origin of isochromosomes. Jour. Genet., **39**: 351–361.

——. 1956. Chromosome botany. London.

——, AND JANAKI AMMAL, E. K. 1945. Chromosome atlas of cultivated plants. London.

——, AND LACOUR, L. F. 1950. Hybridity selection in *Campanula*. Heredity, **4**: 214–248.

——, AND WYLIE, A. P. 1955. Chromosome atlas of flowering plants. London.

DARK, S. O. S. 1932. Chromosomes of *Taxus*, *Sequoia*, *Cryptomeria* and *Thuya*. Ann. Bot., **46**: 965–977.

DELAY, C. 1953. Nombres chromosomiques chez les Cryptogames (1938–1953). Rev. Cytol. Biol. Vegetales, **14**: 59–107.

DOYLE, J. 1945. Naming of the redwoods. Nature, **155**: 254–257.

EAMES, A. J. 1952. Relationships of the Ephedrales. Phytomorphology, **2**: 79–100.

*FAGERLIND, F. 1941. Bau und Entwicklung der *Gnetum*-gametophyten. K. Svenska Vetensk Akad. Handl., **19**: 1–55.

FERNANDES, A. 1936. Sur la caryologie de *Welwitschia mirabilis* Hook. f. Bol. Soc. Brot., **11**: 267–282.

*FLORIN, R. 1932. Die Chromosomenzahlen bei *Welwitschia* und einigen *Ephedra* Arten. Svensk Bot. Tidsk., **26**: 205–214.

——. 1955. The systematics of the Gymnosperms: "A century of progress in the natural sciences. 1853–1953." Calif. Acad. Sci. San Francisco.

FLORY, W. S. 1936. Chromosome numbers and phylogeny in Gymnosperms. Jour. Arnold Arbor., **17**: 83–89.

GARBER, E. 1944. Spontaneous alterations in chromosome morphology of *Nothoscordum fragrans*. Amer. Jour. Bot., **31**: 101–105.

GILLES, A., AND RANDOLPH, L. F. 1951. Reduction of quadrivalent frequency in autotetraploid maize during a period of 10 years. Amer. Jour. Bot., **38**: 12–17.

GRANT, V. 1953. The role of hybridization in the evolution of the leafy-stemmed Gilias. EVOLUTION, **8**: 51–64.

HEILBORN, O. 1934. On the origin and preservation of polyploidy. Hereditas, **19**: 233–242.

*HIRAYOSHI, I., AND NAKAMURA, Y. 1943. (Chromosome number of *Sequoia sempervirens*.) Bot. Zool., **11**: 73–75.

——, NAKAMURA, Y., AND KANO, T. 1943. (Cytological and genetical studies on forest plants. II. Chromosome conjugation in pollen mother cells and pollen fertility of the intermediate black pine (*Pinus densithunbergii* Uyeki).) Seiken Ziho No. 2. 90–96. Vide Pl. Breed. Abstr. 1950, **20**: No. 1937.

*HUNZIKER, J. H. 1953. Número de cromosomas de varias especies sudamericanas de *Ephedra*. Rev. Argent. Agron., **20**: 141–143.

——. 1955. Morfología cromosómica de nueve especies Argentinas del género *Ephedra*. Rev. Investigaciones Agrícolas, **9**: 201–209.

ILLIES, Z. M. 1953. (Selection of multiple seedlings in single plant progenies of *Picea abies*.) Zeit Forstgen. Forstpflanz **2**: 21. Vide Pl. Breed. Abstr. 1953, **23**: No. 1486.

JENSEN, H., AND LEVAN, A. 1941. Colchicine induced tetraploidy in *Sequoia gigantea*. Hereditas, **27**: 220–224.

JOHNSSON, H. 1945. The triploid progeny of the cross diploid x tetraploid *Populus tremula*. Hereditas, **31**: 411–440.

——. 1950. On the C_0 and C_1 generations in *Alnus glutinosa*. Hereditas, **36**: 205–219.

——. 1953. Tree genetics. News letter, **2**: 1–5.

JONES, K. 1954. A consideration of certain aspects of cytotaxonomic investigation in the light of results obtained in *Holcus*. 8th Cong. Int. Bot. Rapp. & Comm. Sect. 9 & 10, pp. 75–77.

KHOSHOO, T. N. 1957. Cytology of conifers III. Partial failure of meiotic spindle in *Cephalotaxus drupacea*. Cytologia, **22**: 80–89.

KIELLANDER, C. L. 1950. Polyploidy in *Picea abies*. Hereditas, **36**: 513–516.

KNABEN, G. 1953. En cytologisk analyse av diploid og triploid *Larix*. Blyttia, **11**: 105–115.

*KRAPOVICKAS, A. M. F. DE. 1955. Complemento cromosomico de especies austroamericanas de *Ephedra*. Rev. Argent. Agron., **21**: 43–45.

LAND, W. J. G. 1913. Vegetative reproduction in an *Ephedra*. Bot. Gaz., **55**: 439–445.

LEVAN, A. 1932. Cytological studies in *Allium* II. The chromosome morphological contributions. Hereditas, **16**: 257–294.

——. 1935. Cytological studies in *Allium* VI. The chromosome morphology of some diploid species of *Allium*. Hereditas, **20**: 289–330.

LEVAN, A., AND EMSWELLER, S. L. 1938. Structural hybridity in *Nothoscordum fragrans* and the origin of terminally constricted chromosomes. Jour. Hered., **29**: 291–294.

LIMA-DE-FARIA, A. 1949. The structure of the centromere of the chromosomes of rye. Hereditas, **35**: 77–85.

——. 1956. The role of kinetochore in chromosome organization. Hereditas, 1956, **42**: 85–160.

LÖVE, A., AND LÖVE, D. 1948. Chromosome number of Northern plant species. Ingolfsprent. Reykjavik.

MANTON, I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge.

MANTON, I., AND SLEDGE, W. A. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Phil. Trans. Roy. Soc. Series B, **238**: 127–185.

MATHEWS, A. C. 1932. Jour. Elisha Mitchell Sci. Soc., **48**: 101. (See Darlington and Janaki Ammal, 1945.)

MEHRA, P. N. 1946a. A study of the karyotypes and the occurrence of diploid male gametophytes in some species of the genus *Ephedra*. Proc. Nat. Acad. Sci. India, **16**: 259–286.

——. 1946b. Method of formation of diploid pollen and the development of male gametophyte in *Ephedra saxatilis* Royle. Jour. Ind. Bot. Soc. (Iyenger Comm. Vol.) pp. 121–132.

——, AND KHOSHOO, T. N. 1956a. Cytology of conifers. I. Jour. Genet., **54**: 165–180.

——. 1956b. Cytology of conifers. II. Jour. Genet., **54**: 181–185.

MIKI, S., AND HIKITA, S. 1951. Probable chromosome number of fossil *Sequoia* and *Metasequoia* found in Japan. Science, **113**: 3–4.

MÜNTZING, A. 1933. Hybrid incompatibility and the origin of polyploidy. Hereditas, **18**: 33–55.

——. 1936a. The evolutionary significance of autopolyploidy. Hereditas, **21**: 263–378.

——. 1936b. The chromosomes of a giant *Populus tremula*. Hereditas, **21**: 383–393.

*NAVASHIN, M. 1932. The dislocation hypothesis of evolution of chromosome numbers. Zeit. Ind. Abst. u. Vererb., **63**: 224–231.

OSBORN, A. 1941. An interesting hybrid conifer: *Cupressocyparis leylandii*. Jour. Roy. Hort. Soc., **66**: 54–55.

PLATT, R. 1953. A pocket guide to the trees. Pocket Books, Inc. New York. Cardinal Edition.

*RESENDE, F. 1937. Planta, **26**: 757–807. Vide Darlington and Janaki Ammal, 1945.

*RHOADES, M. M. 1940. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics, **25**: 483–513.

RODGER, G. J. 1953–1954. Annual report of the Forestry and Timber Bureau, Commonwealth of Australia for 1953–1954. Vide Pl. Breed. Abstr. 1956, **26**: No. 2861.

ROSS, J. R., AND DUNCAN, R. E. 1949. Cytological evidences of hybridization between *Juniperus virginiana* and *J. horizontalis*. Bull. Torr. Bot. Club, **76**: 414–429.

SAKAI, K., AND SUZUKI, Y. 1955a. Studies on competition in plants. II. Competition between diploid and autotetraploid plants of Barley. Jour. Genet., **53**: 11–20.

——. 1955b. Studies on competition in plants. V. Competition between allopolyploids and their diploid parents. Jour. Genet., **53**: 585–590.

SAX, H. J. 1932. Chromosome pairing in *Larix* species. Jour. Arnold Arbor., **13**: 368–373.

SAX, K., AND BEAL, J. M. 1934. Chromosomes of Cycadales. Jour. Arnold Arbor., **15**: 255–262.

——, AND SAX, H. J. 1933. Chromosome number and morphology in conifers. Jour. Arnold Arbor., **14**: 356–375.

SEARS, E. R. 1952a. Misdivision of univalents in common wheat. Chromosoma 1952, **4**: 535–550. Vide Pl. Breed. Abstr. 1952, **22**: No. 1773.

——. The behavior of isochromosomes and telocentrics in wheat. Chromosoma, **4**: 551–562. Vide Pl. Breed. Abstr. 1952, **22**: No. 1774.

STEBBINS, G. L., JR. 1938. Cytological characteristics associated with the different growth habits in the dicotyledons. Amer. Jour. Bot., **25**: 189–198.

——. 1947. Types of polyploids: their classification and significance. Adv. Genet., **1**: 403–429.

——. 1948. The chromosomes and relationships of *Metasequoia* and *Sequoia*. Science, **108**: 95–98.

——. 1950. Variation and evolution in plants. New York.

STEERE, W. C. 1954. Chromosome number and behavior in arctic mosses. Bot. Gaz., **116**: 93–133.

*——, ANDERSON, L. E., AND BRYAN, V. S. 1954. Chromosome studies on California mosses. Mem. Torr. Bot. Club, **20**: 1–76.

STIFF, M. L. 1951. A naturally occurring triploid Juniper. Va. J. Sci., **2**: 317. Vide Pl. Breed. Abstr. 1952, **22**: No. 1474.

SUNDAR RAO, Y. 1950. Karyosystematic studies in Helobiales. Proc. Nat. Inst. Sci., **19**: 563–581.

SYRACH-LARSEN, C., AND WESTERGAARD, M. 1938. A triploid hybrid between *Larix decidua* Mill. and *L. occidentalis* Nutt. Jour. Genet., **36**: 523–530.

TAKHTAJAN, A. L. 1953. Phylogenetic principles of the system of higher plants. Bot. Rev., **19**: 1–45.

TATUNO, S. 1949. Zytologische Untersuchungen uber die Lebermoose von Japan. Jour. Sci. Hiroshima Univ. Ser. B, **4**: 73–115.

TJIO, J. H., AND LEVAN, A. 1950. The use of oxiquinoline in chromosome analysis. Ann. Estac. Experi. Aula Die, **2**: 21–64.

VERMA, S. C. 1956. Cytology of *Ophioglossum*. Curr. Sci., **25**: 398–399.

YASUI, K. 1946. (On polyploidy in the genus *Sequoia*.) Jap. Jour. Genet., **21**: 9–10. Vide Pl. Breed. Abstr. 1951, **21**: No. 3045.

ZINNAI, I. 1952. (Tetraploid plants of Japanese Red *Pinus-Pinus densiflora* S. et. Z.-discovered in transplant beds.) Jour. Jap. For. Soc., **34**: 185–187. Vide Forestry Abstracts 1953, **14**: 402.

ZINNAI, I., AND CHIBA, S. 1951. (Naturally occurring tetraploids of *Cryptomeria japonica*) Ikushugaku Zasshi Jap. J. Breed., **1**: 43–46. Vide Pl. Breed. Abstr. 1952, **22**: No. 1473.

SPECIATION IN BUTTERFLIES OF THE *PAPILIO GLAUCUS* GROUP. I. MORPHOLOGICAL RELATIONSHIPS AND HYBRIDIZATION

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Received April 10, 1958

Speciation is represented by the spectrum of events that occur during the establishment of sexual isolation between populations. This reproductive isolation may be actual or only potential depending on whether the populations are, respectively, sympatric or allopatric in the true biological sense. Thus if populations of two or more animals co-occur in space and in time so that at least some individuals from each intermingle without intermating, then there is actual sexual isolation. But when populations which have been named as distinct species solely on the basis of their morphological discontinuity do not co-occur in this manner, then it is assumed that there is potential sexual isolation such that they would not freely hybridize should they meet in nature. Moreover, in order for closely related but sexually isolated populations to enter the range of each other, there must be little or no competition for the same environmental resources because this would almost inevitably lead to the survival of only one species through differential reproduction.

With these considerations in mind, it was decided to investigate the ecological and phylogenetic relationships of the tiger swallowtail butterflies comprising the *Papilio glaucus* group. These butterflies form a morphologically compact group of closely related species limited to North America with representatives occurring from the Atlantic coast to the Pacific and from Alaska to southern Mexico (see fig. 1). The purpose of this paper is to present new findings on the morphological relationships and to give evidence for natural hybridization. A separate paper (Brower, 1959) will describe their geographic distribution in relation to the distribution of possible foodplants, and another will discuss autecological differences in areas of co-occurrence in relation to interspecific sexual behavior.

It has generally been considered that this group contains six species: *Papilio alexiares* Hopffer, *P. eurymedon* Lucas, *P. glaucus* Linné, *P. multicaudatus* Kirby, *P. rutulus* Lucas, and *P. pilumnus* Boisduval (Rothschild and Jordan, 1906). As this paper will demonstrate, and as Edwards briefly noted (1897), *P. pilumnus* should be removed from the *P. glaucus* group and placed with *P. palamedes* Drury and *P. troilus* Linné, another group of North American swallowtails related to the *glaucus* group. *P. glaucus* occurs generally in eastern North America and in the northern part of its range it extends across Canada and northwestward to Alaska, its northern limit being roughly coincident with that of the Hudsonian biotic province as defined by Dice (1943). *P. eurymedon, P. multicaudatus,* and *P. rutulus* replace *P. glaucus* in the western mountain area and extend from southern British Columbia southward. *P. alexiares* is known only from the mountainous regions of eastern Mexico. Certain areas of overlapping distribution have been found: 1) the three "western species" co-occur throughout most of their range in the western United States; 2) in southern British Columbia the three western species occur with *P. glaucus;* 3) *P. multicaudatus* is more widespread than either *P. rutulus* or *P.*

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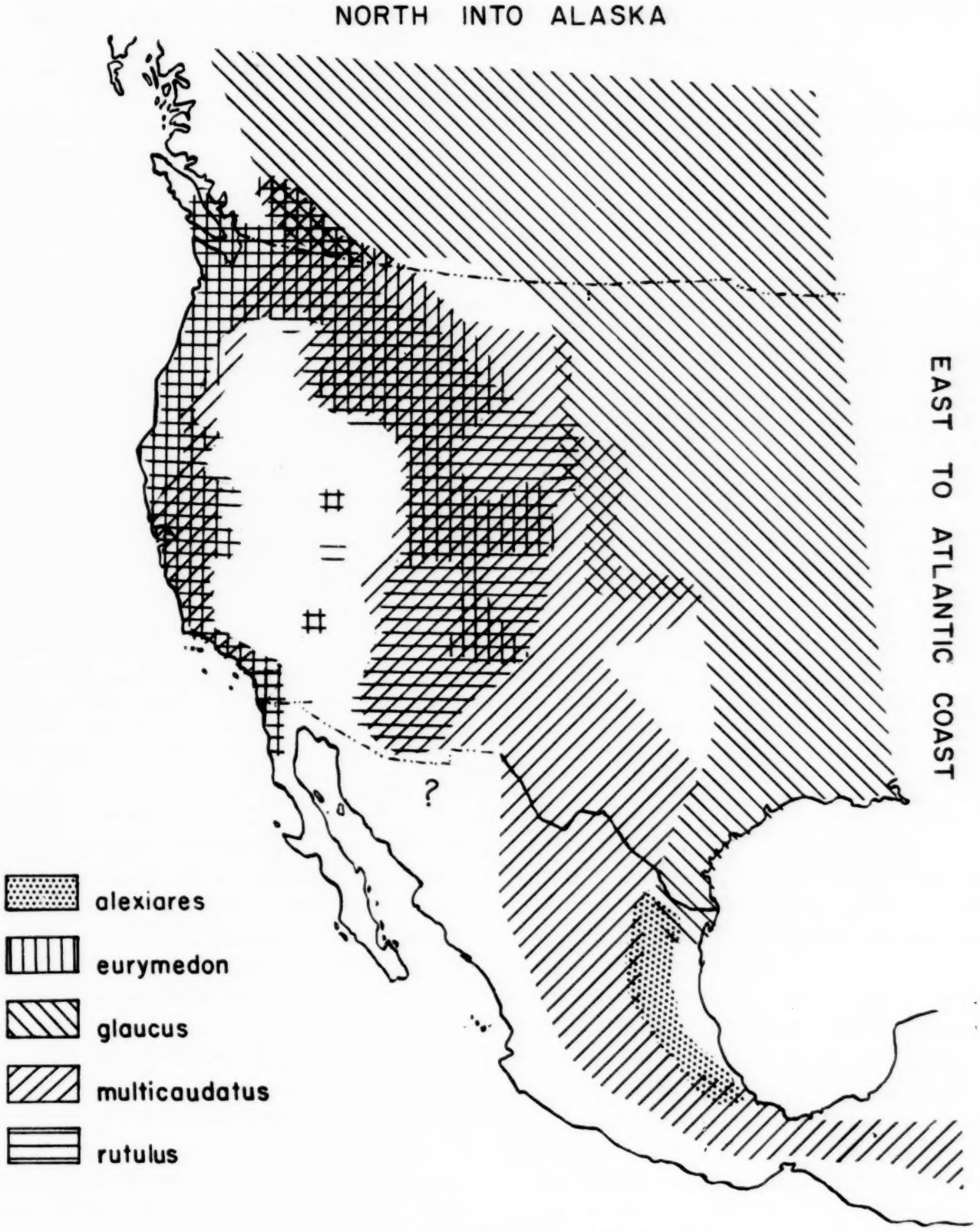

FIG. 1. Known distribution of the *Glaucus* group.

eurymedon and in several areas along its eastern fringe it overlaps the western fringe of *P. glaucus;* 4) there is evidence for the overlap of *P. glaucus* and *P. rutulus* in western Nebraska and in the Black Hills of South Dakota; 5) in eastern Mexico, *P. multicaudatus* and *P. alexiares* occur together and in northeastern Mexico both occur with *P. glaucus* (see figure 1).

MARGINAL OVERLAPPING AND HYBRIDIZATION BETWEEN *P. glaucus* AND *P. rutulus*

The similar appearance of the adults of *P. glaucus* and *P. rutulus* has led many authors to regard them as conspecific, while others have maintained that they are separate. This controversy has never

TABLE 1. *Frequency of* Papilio glaucus, P. rutulus, P. eurymedon, P. multicaudatus, *and "undetermined" specimens (possible* P. glaucus × P. rutulus *hybrids) in British Columbia and South Dakota as found in museums and private collections*

Sample location	*glaucus*	*rutulus*	"undet."	*eurymedon*	*multicaudatus*
B. C. Area No. 1 (Cranbrook)	21	0	4	7	0
B. C. Area No. 2 (Kootenay Lk. Rgn. & Arrow Lks. Rgn.)	9	18	1	28	3
B. C. Area No. 3 (Okanogan Valley)	3	36	7	12	22
B. C. Area No. 4 (Lower Fraser R. Valley & Vancouver Island)	0	26	0	29	0
B. C. Area No. 5 (Mid Fraser R. Valley & S. Thompson R. Drainage System)	25	7	0	7	7
B. C. Area No. 6 (Regions N. of B. C. Area Nos. 1–5)	15	0	0	0	0
South Dakota (Black Hills)	39	2	18	0	1

Note especially the increase in relative frequency of *P. rutulus* from east to west (B. C. Area Nos. 1 to 4) and its decrease from south to north (B. C. Area 4 to 5 and 6). Note also that where *P. glaucus* and *P. rutulus* are both proportionately common (B. C. Area Nos. 2 and 5), the "undetermined" specimens are few or absent, but that where *P. glaucus* and *P. rutulus* are both present, but one is rare, "undetermined" specimens are more frequent (B. C. Area No. 3).

been settled and is due largely to four factors. First, there is a complete lack of quantitative data comparing the geographic variation of individual adult characters. Second, there occur in populations of each species, no matter where they are from, individuals which possess an adult character or characters typical of the "wrong" species. Third, lepidopterists have erroneously accepted one "key" character difference of several differences originally described (distinct spots *versus* continuous band on the underside of the forewing margin), and this simply does not take the geographic variation of the two species into account. Fourth, there has been an over-emphasis on adult pattern and coloration, and no one has studied whether or not larval and genital characters intergrade geographically.

The method of investigating the relationship of these two butterflies was twofold. First, as discussed below under the section on intra-group relationships, larval pattern and male genital characters were studied. Second, adult specimens were compared from various localities in southwestern Canada and the western United States with respect to another of the historically noted differences, the presence of orange coloration (*P. glaucus*) or its absence (*P. rutulus*) in the submarginal lunules on the underside of the hindwings. A specimen was regarded as *P. glaucus,* if the second through fourth submarginal lunules had a substantial area of orange scaling. If these three lunules had no orange in them and were therefore completely yellow, the specimen was scored as *P. rutulus.* Occasionally the second through fourth lunule had a very small central patch of orange scales. These specimens were considered "undetermined." All specimens regarded as *P. glaucus, P. rutulus,* or "undetermined" were seen by me with the following exceptions which were confirmed by letter: 4 *P. rutulus* from British Columbia Area

TABLE 2. *Locality, inclusive dates of capture, collectors, and present location data for male and female specimens of the* Papilio glaucus *group and* P. troilus *group*

Specimen reference numbers to genitalia in figures 3–6 and listed in table 4 appear in parentheses.

	Sp.	Total	Locality, dates, collectors; location
Alaska	g	45 ♂♂, 2 ♀♀	Big Delta, College (69–73, 75–78), Ester (74) 28 May–26 Jun 51–52 PB & GR, JM, WM; CNC, YPM*
Alberta	g	16 ♂♂, 9 ♀♀	Crowsnest Pass, Didsbury (65–68), Elkwater, Elkwater Lk., Waterton, Waterton Lks. 1 Jun–18 Jul 03–56 CG, JM, JP, ES; AMH*, CNC
British Columbia			
Area No. 1	e	6 ♂♂, 1 ♀	Cranbrook (g 49–52, 55–58; ? 53–54)
	g	18 ♂♂, 3 ♀♀	2 May–19 Jun 09–17 CG; CFD, AMH*
	?	4 ♂♂	
Area No. 2	e	24 ♂♂, 4 ♀♀	Duncan R., Kaslo, Nelson, Robson (r 41–45; ? 46), "Arrowhead Lk."
	g	9 ♂♂	
	m	3 ♂♂	7 Apr–15 Jun 00–35 WB, JWC, JMcD; AEB,
	r	14 ♂♂, 4 ♀♀	CFD, AMH*, CNC, USM
	?	1 ♂	
Area No. 3	e	11 ♂♂, 1 ♀	Vernon, Okanogan, Summerland, Naramata, Okanogan Falls, Keremeos, Oliver, Osoyoos
	g	2 ♂♂, 1 ♀	
	m	19 ♂♂, 3 ♀♀	13 May–21 Jul 03–53 AG, BG, CG, DH, JJ, JM,
	r	34 ♂♂, 2 ♀♀	JMc, JMcD, GW; RLC, RJF,
	?	6 ♂♂, 1 ♀	AMH, CNC, USM
Area No. 4	e	22 ♂♂, 7 ♀♀	Harrison Hot Springs, Agassiz, Haney, Vancouver, N.
	r	19 ♂♂, 7 ♀♀	Vancouver, Brakendale; Oyster R., Courtenay, Comox, Wellington, Duncan, Maple Bay, Sidney, Victoria, Goldstream
			28 Apr–19 Jul 85–56 RNC, WC, WD, HE, F, JF, RG, J, AL, CL, JMcD, EM, EO, HP, GT, FW, CY; CFD, DCF, AMH, CNC, LAM, USM, YPM
Area No. 5	e	7 ♂♂	Lillooet (r 47–48), Seton Lk., Pavillion, Pavillion Lk.,
	g	19 ♂♂, 6 ♀♀	D'Arcy, Anderson Lk.; Kamloops, Heffley Cr., Rayleigh, Notch Hill, Salmon Arm, Enderby
	m	6 ♂♂, 1 ♀	
	r	6 ♂♂, 1 ♀	24 May–30 Jun 21–39 RLC, AD, JJ, JMcD, WHP, GW; RLC, AMH*, CNC
Area No. 6	g	10 ♂♂, 5 ♀♀	Clinton, 100 Mile House, Canim Lk., Quesnel, Stanley, Mt. Robson
			12 Jun–3 Jul 21–38 F, JJ, GW; AMH, CNC
California			
San Benito Co.	r ?	1 ♀	Pinnacles National Monument 24 Jul 49 CM; LPB
Colorado			
Boulder Co.	e	n ♂♂ & ♀♀	Boulder, Boulder Can., Buckingham Pk., Eldora, Flagstaff Mt., Fourmile Can., Glendale, James Cr. nr. Jamestown, Left Hand Can., Nederland, Rd. to Rainbow Lks., Rollinsville, Science Lodge nr. Ward, So. St. Vrain Can., Spring Gulch, Sunbeam Gulch nr. Crisman (e 99–108); m 1–10; r 21–30)
	m	n ♂♂ & ♀♀	
	r	24 ♂♂, n ♀♀	15 May–15 Aug 33–55 LB, DE, CR, PR; LPB*, YPM

TABLE 2—*Continued*

	Sp.	Total	Locality, dates, collectors; location
Florida			
Highlands Co.	g	9 ♂♂	Parker Islands (g 127–135; pal 125–126; t 123)
	pal	2 ♂♂	4 Mar–24 Jul 55–56 RA, LB; LPB*
	t	1 ♂	
Illinois			
Cook Co.	g	3 ♂♂	Chicago (64, 91, 98)
			reared 55–56 ED; LPB*, PMS*
Jersey Co.	g	8 ♂♂	Elsah (89–90, 92–97)
			17–25 Apr 42 CR; LPB*
New Jersey			
Morris Co.	t	1 ♂	Chatham Township (124)
			10 Sep 56 LB; LPB*
N. W. Territory	g	13 ♂♂, 10 ♀♀	Fort Simpson
			3 Jun–17 Jul 50 DW; CNC
	g	22 ♂♂, 6 ♀♀	Fort Smith
			4–26 Jun 50 JW; CNC
Mexico			
Nuevo Leon	a	2 ♂♂	Victoria (119–120)
			30 May–11 Jun 41 —; AMH*
Vera Cruz	pil	2 ♂♂	Orizaba (121–122)
			——— —; AMH*
South Dakota			
Pennington Co.	g	39 ♂♂	Black Hills (g 79, 84, 85, 87; ? 80–83, 86, 88)
	m	1 ♂	13–17 Jun 54 LB; LPB*
	r	2 ♀♀	
	?	17 ♂♂, 1 ♀	
Utah			
Salt Lake Co.	e	n ♂♂ & ♀♀	Big Cottonwood Can. (e 109–118; m 11–20; r 31–40)
	m	n ♂♂ & ♀♀	22 May–22 Jul 48–55 LB, WP; LPB*
	r	67 ♂♂, n ♀♀	
Washington			
Okanogan Co.	r	15 ♂♂, 7 ♀♀	Alta Lk., Brewster, Camp Gilbert, Gold Cr., Pateros, Patterson Lk., Salmon Meadows
			13 May–26 Jul 50–54 AA, JH; CNC, YPM

Abbreviations for Tables 2 and 3:

Species: a = *Papilio alexiares garcia;* e = *P. eurymedon;* g = *P. glaucus;* m = *P. multicaudatus;* pal = *P. palamedes;* pil = *P. pilumnus;* r = *P. rutulus;* t = *P. troilus;* r ? = *P. glaucus*-like *P. rutulus;* ? = "undetermined" (see text).

Collectors: AA = A. Anderson; RA = R. Archbold; LB = L. P. Brower; PB = P. F. Bellinger; WB = W. Barnes; JCC = J. C. Chamberlain; JWC = J. W. Cockle; RLC = R. L. Chermock; RNC = R. N. Chrystal; WC = W. R. Carter; AD = A. A. Dennys; ED = E. Dluhy; WD = W. Downes; DE = J. D. Eff; HE = H. Edwards; WE = W. H. Evans; JF = J. Fletcher; F = Fraser; AG = A. N. Gartrell; BG = C. deB. Greene; CG = C. B. Garrett; RG = R. Guppy; DH = D. F. Hardwick, JH = J. C. Hopfinger, JJ = J. K. Jacob, J = Johnson; AL = A. W. Lindsey; CL = C. Livingston; DL = D. J. Lennox; CM = C. D. MacNeill; JM = J. E. H. Martin; EM = E. Mason; WM = W. R. Mason; JMcD = J. McDunnough; JMc = J. R. McGillis; EO = E. T. Owen; JP = J. H. Pepper; WLP = W. L. Phillips; WHP = W. H. A. Preece; GR = G. W. Rawson; CR = C. L. Remington; PR = P. S. Remington; ES = E. E. Sterns; GT = G. W. Taylor; GW = G. S. Walley; JW = J. B. Wallis; DW = D. P. Williams; FW = F. H. Wolley-Dod; CY = C. H. Young; CS = Canadian Insect Survey.

Present Locations: AEB = A. E. Brower; LPB = L. P. Brower; RLC = R. L. Chermock; CFD = C. F. dos Passos; DCF = D. C. Ferguson; RJF = R. J. Ford; PMS = P. M. Sheppard; AMH = American Museum of Natural History; CNC = Canadian National Collection; LAM = Los Angeles Museum; USM = United States National Museum; YPM = Yale Peabody Museum.

* = collection in which specimens in figures 3–6 and table 4 are located.

No. 3 and 2 *P. glaucus* from B. C. Area No. 5. Because of the great confusion that has existed in the literature concerning the determination of *P. glaucus* and *P. rutulus,* it is quite probable that the relative frequencies of these two butterflies (and "undetermined" specimens) in private and institutional collections are unbiased. It was assumed that no confusion existed between these and *P. eurymedon* and *P. multicaudatus,* but the frequencies of the latter two are probably biased and therefore valid frequency comparison is limited to *P. glaucus, P. rutulus,* and "undetermined" specimens. Data for all individuals studied are summarized in table 2.

British Columbia specimens gathered from numerous localities by several collectors from 1885 to 1956 were separated by geographic area into six groups. The first group is from Cranbrook, located on the western edge of the Kootenay River Valley about 40 miles north of the U. S. border (British Columbia Area No. 1). This valley is the first of several running north and south that are encountered as one proceeds westward from the Continental Divide. The second valley system to the west (Area No. 2) includes localities along the Duncan River, the northern half of Kootenay Lake, and along the river branch that flows westward from this lake into the Columbia River near Robson, as well as the Arrow Lakes region. Area No. 3, approximately 90 miles further west, includes several localities in the Okanogan Valley from Vernon to Osoyoos. Area No. 4, still further west at approximately the same latitude, consists of several localities in the southern part of the valley of the Fraser River and on Vancouver Island. On an average of about 80 miles north of these four regions is Area No. 5 which includes the middle part of the Fraser River Valley near Lillooet as well as the southern part of the Thompson River drainage system in the vicinity of Kamloops and Salmon Arm. Area No. 6 contains several localities north of the five areas just enumerated.

Three interesting facts emerge from a study of the lunule coloration of the specimens collected from these six areas in British Columbia (see table 1). First, there is a striking geographic replacement of *P. glaucus* by *P. rutulus* proceeding west from the southeastern border of the province (Area Nos. 1 to 4), but to the north *P. rutulus* gives way again to *P. glaucus* (Area Nos. 5 and 6). Second, if it is assumed (a) that the "undetermined" specimens are hybrids between the two species, (b) that mating between them is at random, and (c) that the hybrids possess normal viability, then in two out of the three areas where both *P. glaucus* and *P. rutulus* occur, the number of hybrids found is significantly less than the number expected. In Area No. 2 the expected number of hybrids is 12.4 and the probability (calculated from the binomial distribution with $p = 12.4/28$) of obtaining less than or equal to 1 is much less than .001. Similarly, in Area No. 5 the expected number is 10.9 and the probability (calculated from the binomial distribution with $p = 10.9/32$) of obtaining 0 is much less than .001. Differing from these two areas is Area No. 3 where the observed and expected are nearly identical, being 7 and 6.5, respectively. Third, in the two areas where there is a deficiency of hybrids (Area Nos. 2 and 5), the least common species is from 3.1 to 4.6 times more common than it is in Area No. 3 where the observed and expected frequency of hybrids are nearly equal. A test of the null hypothesis that there is no difference in the proportion of hybrids to the least common species in Area No. 3 versus the proportion of hybrids to the least common species in Area No. 2 added to Area No. 5 gave a chi-squared value (with the Yates correction factor) of 9.55. The difference in proportions is thus highly significant (p less than .005, with 1 degree of freedom). One possible interpretation of these data is that natural selection has

resulted in barriers to hybridization in areas where both species are present in substantial relative numbers, but not in that area where only one species is common.

In contrast to these three British Columbia areas where the observed numbers of hybrids are less than or equal to the expected, there is a highly significant excess in the number of hybrids found in the Black Hills of South Dakota and in B. C. Area No. 1 (see table 1). The expected number in the Black Hills is 5.5 and the observed is 18.0. The probability of obtaining such a deviation as this is much less than .001 (chi-squared with Yates correction = 28.9; 1 degree of freedom). The interpretation of this will be discussed below.

This lunule coloration character was also studied in series of specimens from several other localities. In a series of 22 specimens from Okanogan County in Washington none had orange in its lunules. Thus directly south of British Columbia Area No. 3 along a continuation of the same valley system, the population seems to be pure *P. rutulus*. A similar situation is seen in the lower part of the Fraser Valley about 100 miles south of Area No. 5 and on Vancouver Island where each of 26 specimens was determined as pure *P. rutulus* (Area No. 4). A series of 25 specimens from southwestern Alberta on the east side of the Continental Divide, adjacent to British Columbia Area No. 1 where several "undetermined" specimens were found, all proved to be *P. glaucus*. Further to the north, 28 specimens from Fort Smith on the Alberta-North West Territory border were all *P. glaucus*, as was the case for 23 from Fort Simpson, N. W. T. From the Fairbanks region of Alaska, 47 specimens also were determined as *P. glaucus*. The only *P. rutulus* specimen known to me from Alaska is one which Barnes and McDunnough (1916) described from a series of several collected at Chatanika and which are now in the U. S. National Museum. These authors correctly concluded that it is *P. rutulus*, while the rest are *P. glaucus canadensis*. However, since this is a lone specimen and disagrees strikingly in size, color, and pattern from all the others in the large series from this entire region, it seems likely that the locality label on the *P. rutulus* is erroneous.

In July, 1955, 67 specimens, again all *P. rutulus*, were collected in Salt Lake County, Utah. However, samples from two other regions each included a single specimen which keyed to an unexpected category: (1) in a series of 24 specimens from Boulder County, Colorado, where all were expected to be pure *P. rutulus*, one (No. 22) had sufficient orange in the lunules to be placed as *P. glaucus*, and (2) although definite quantitative data are lacking, of the numerous specimens of *P. rutulus* which I have seen from California, one sent to me by C. D. MacNeill from San Benito County also fell into the *P. glaucus* category. From this it is apparent that populations of one species occasionally contain individuals which on the basis of lunule coloration resemble the other. However, the frequency of these in all of the western North American areas studied is very low.[2] Thus *P. glaucus* and *P. rutulus* exhibit mutual geographic replacement in western North America with a narrow zone of sympatry in which varying degrees of hybridization seem to occur.

Intra-Group Relationships: Larval Color Pattern and Pupal Morphology

The first method used in studying intra-group phylogeny in the *P. glaucus* group was a comparison of the eye-spots on the third thoracic segment of the fifth instar larvae. This comparison was limited due to lack of material. It was not possible to include *P. alexiares* because its larva is

[2] In southeastern Canada, northeastern United States, and in individuals of the spring brood as far south as Georgia, the orange coloration in these lunules is often much reduced, and in rare cases absent.

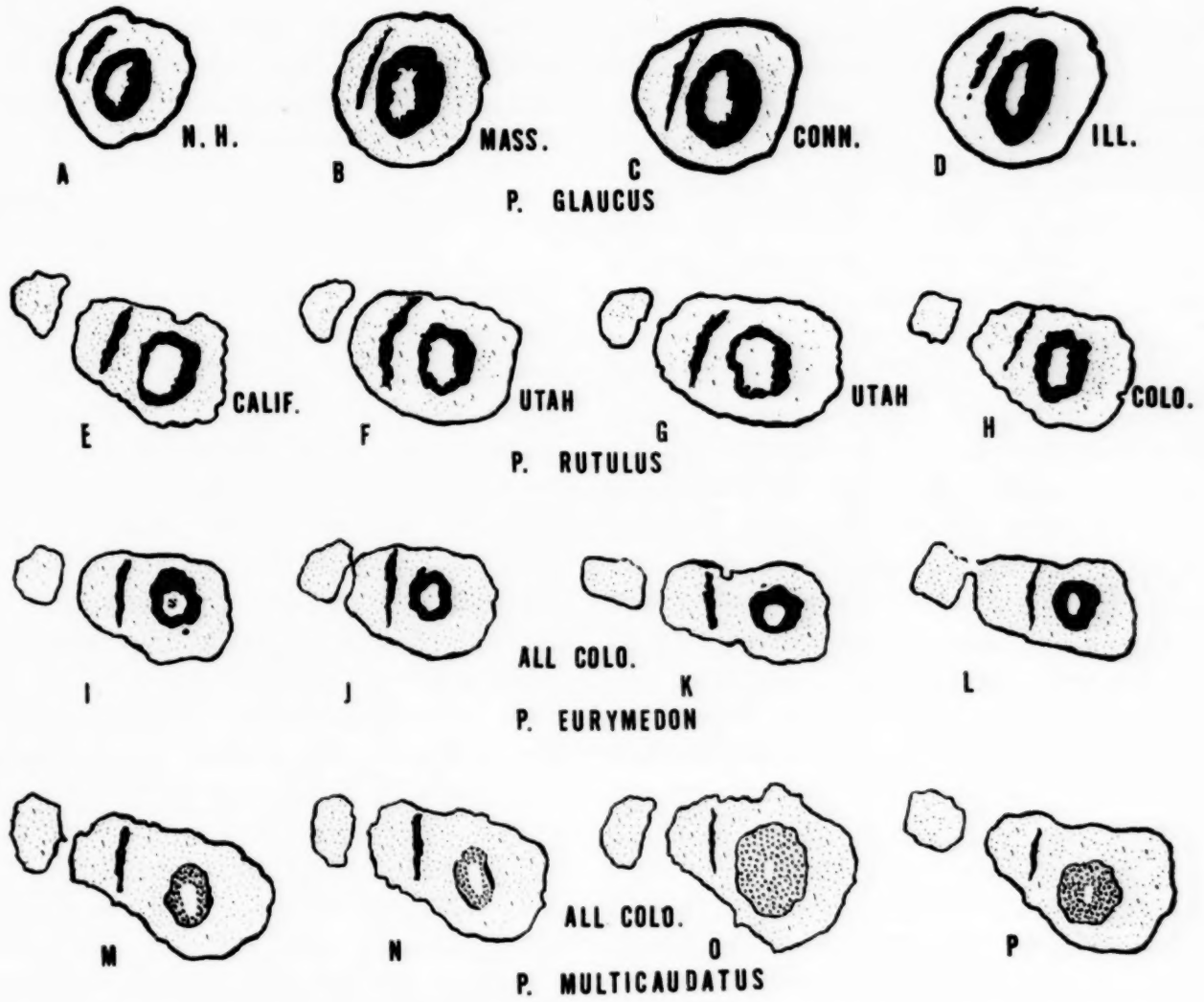

FIG. 2. Right "eye-spot" on dorsum of 3rd thoracic segment of 5th instar larvae in *P. glaucus* group from various geographic areas. Note that *P. glaucus* has a single element while the three western species each have a double element. Data are in table 3.

unknown. Drawings were made from preserved larvae under a binocular dissecting microscope in freehand by John G. Coutsis, and no particular care was taken to give absolute size dimensions, because these were noted to vary according to how early or late in the fifth instar the larvae were preserved. Since the interest here is mainly in a comparison of the shape, number, and position of the various elements of the eye-spots, it is felt that the omission of this factor does not affect the conclusions. Further comparisons of series of larvae from several different localities will be needed to confirm the conclusions of this study.

1. *P. palamedes, P. pilumnus, P. troilus*

Although I have not been able to locate any larvae of *P. pilumnus,* the detailed description of a mature larva given by Schaus (1884) almost certainly places it with *P. troilus* and *P. palamedes,* as noted briefly by Edwards (1897). Both *P. pilumnus* and *P. troilus* larvae have a yellow line running along each side of the body at the level of the spiracles, and both have a series of 7 blue spots on the second through eighth abdominal segments just below this yellow line. Neither of these characters is present in *P. eurymedon, P. glaucus, P. multicaudatus,* or *P. rutulus.* Schaus's description of the eye-spot on the third thoracic segment also strongly suggests the close relationship of *P. pilumnus* and *P. troilus.* Furthermore, he mentions that the pupa of *P. pilumnus* is pale green or pink. As far as I know, no pupae of the species of the *P. glaucus* group have been found which are uniformly green, whereas both *P. troilus* and *P. palamedes* have pupae of

this color phase (Scudder, 1889). One pupal phase of *P. eurymedon* has a considerable area colored pale green (Edwards, 1874), but there are two lateral stripes and one dorsal stripe running its entire length which are brownish in color and very conspicuous (personal observation). Pink pupae are unknown in the *P. glaucus* group.

In Mosher's (1916) keys for separating pupae of members of the *P. glaucus* group from those of the *P. troilus* group, the first character difference mentioned is "body surface without distinctly carinate ridges," and "body surface with distinctly carinate ridges," respectively, for the two groups. This key was based on a study including *P. eurymedon, P. glaucus, P. multicaudatus,* and *P. rutulus* for the *P. glaucus* group, and *P. palamedes* and *P. troilus* for the *P. troilus* group. In describing the morphology of the pupa of *P. pilumnus,* Schaus mentioned that it is laterally ridged. Thus, running Schaus's description in Mosher's key also leads one to conclude that *P. pilumnus* belongs in the *P. troilus* group.

2. *P. eurymedon, P. glaucus, P. multicaudatus, P. rutulus*

The fifth instar larvae of the *P. glaucus* group which I examined or determined from the literature are listed in table 3. Without exception, all these specimens, or their descriptions, resembled the drawings of the four respective species in figure 2, with regard to the number and general shape of the elements comprising the eye-spot character on the third thoracic segment. Although it is unfortunate that no northwestern larvae of *P. glaucus* were available, the fifteen larvae from Ontario and Quebec, which were typically *P. glaucus*-like, indicate that *P. rutulus*-like markings are not characteristic of northeastern populations, even though adults from this area do occasionally resemble adult *P. rutulus* in their lunule coloration. On the basis of these larval data, it appears that the three western species (*P. eurymedon, P. multicaudatus, P. rutulus*) are more closely allied to each other than any one of them is to *P. glaucus,* and that *P. rutulus* and *P. eurymedon* are the most similar.

Intra-Group Relationships: Male Genital Morphology

The second means of comparing all the species was through a study of the male genitalia of 135 specimens from various areas listed in table 2. The genitalic preparations were begun by cutting off the end of the abdomen and placing each in an individual dish of 10% KOH solution at room temperature for from 48 to 72 hours. At the end of this period, each preparation was placed in 30% ethyl alcohol where the gut contents were cleared out. At the same time the scales of the valvae were removed with a small brush and a flat dissecting needle. Each preparation was then placed in a 1 dram vial of 95% alcohol for a few days, after which each was run through 100% alcohol, clove oil, and then xylene, for about 10–20 minutes each. The next step was to remove the aedeagus from its enclosing membranes and then to dissect both valvae and the annelus intact from the rest of the genitalia. After this, the valvae and annelus were temporarily mounted on a slide in gum damar in a spread position with the armature of the valvae facing the cover slip. This was then pressed down to push parts on the armature into a position parallel with the slide rather than perpendicular or semi-perpendicular to it. This procedure was especially necessary in the armature of *P. glaucus* where some parts are more curved into the "perpendicular" position than in any of the other species. The purpose of pressing the valvae in this way was to facilitate photography. Extreme care was taken not to break the spines of the armature, or to distort excessively the valvae themselves. When thus mounted, the preparation was photographed, de-mounted, placed successively in xylene, 100% alcohol, 95% alcohol, and then stored permanently in 80% alcohol with 4% glycerine.

TABLE 3. *Locality, dates of preservation, collectors, and present location data for 5th instar larvae of the* P. glaucus *group*

	Sp.	Fig.	Total	Locality, dates, collectors, location
California	r	E	4	La Tuna Can. (reared from egg)
Los Angeles Co.				— 50–51 WE; YPM
	e	—	—	H. Edwards (1874)
Colorado	m	M–P	65	Left Hand Can.
Boulder Co.				Aug–Sep 55 LB; LPB (8 preserved)
	r	H	2	Boulder
				6 Sep 55 LB; LPB
	r	—	1	Chicken Ranch Gulch
				3 Sep 53 CR; YPM
	e	I–L	56	Left Hand Can.
				Aug–Sep 55–56 LB; LPB (8 preserved)
Connecticut				
Litchfield Co.	g	C	1	2 mi. w. of Bantam
				14 Aug 52 CR; YPM
New Haven Co.	g	—	4	West Haven
				–Sep 56 LB; —
Illinois				
Jersey Co.	g	D	1	Elsah
				1 Jun 46 CR; YPM
Massachusetts				
Middlesex Co.	g	B	1	Cambridge
				27 Aug 48 CR; YPM
Mexico	m	—	—	Schaus (1884); Seurat (1898); Ruelas (1925)
New Hampshire				
Coos Co.	g	A	1	Jefferson
				20 Aug 50 DL; YPM
New Jersey				
Morris Co.	g	—	5	Chatham Township
				10 Sep 55 LB; —
Ontario and Quebec	g	—	15	Kimburn, Ont.; Lac Phillippe, Que. (reared from eggs of 4 ♀♀)
				18 Jul–7 Aug 54 CS; CNC
Utah				
Salt Lake Co.	r	F–G	2	Big Cottonwood, Can.
				1 Sep 55 LB; LPB.

For abbreviations see table 2.

Throughout the process, each dissection had its own number, and that number now accompanies the butterfly from which the genitalia came, the genitalic preparation, and the figures of the genitalia in this paper.

In order to photograph the eighth tergite with its associated superuncus, the genitalia, without the valvae, were also mounted in damar on a slide, but in this case the cover slip was held up with glass chips to prevent any flattening of the structure.

Photographs were taken on 35 mm Kodak Panatomic X film at 1/25 of a second with a Kine Exakta VX through a compound microscope with a Bausch and Lomb 48 mm micro tessar lens set at F-16, and then enlarged to 8×. The photographs were then used as a basis for

TABLE 4. *Morphological measurements and ratios*

Spn. no.	T	SU	SU/T	V	FW	V/FW	Spn. no.	T	SU	SU/T	V	FW	V/FW
m 1	24.0	14.0	.583	6.38	52	.123	g 50	19.0	9.0	.474	5.13	45	.114
m 2	21.0	15.0	.714	6.38	51	.125	g 51	18.0	10.0	.556	4.75	45	.106
m 3	20.0	17.5	.875	6.88	57	.121	g 52	19.0	8.0	.421	4.50	38	.118
m 4	21.5	16.5	.767	6.88	54	.127	g 53	19.0	8.5	.447	4.88	44	.111
m 5	21.5	16.0	.744	6.88	53	.130	g 54	21.0	8.5	.405	4.63	44	.105
m 6	25.5	16.0	.627	7.38	60	.123	g 55	19.0	9.0	.474	5.00	40	.125
m 7	25.5	17.5	.686	7.00	56	.125	g 56	21.0	9.5	.452	4.75	44	.108
m 8	28.5	16.5	.579	7.00	61	.115	g 57	19.0	9.5	.500	5.00	44	.114
m 9	23.5	15.0	.638	6.50	52	.125	g 58	17.5	9.0	.514	4.38	43	.102
m 10	22.0	15.5	.705	6.63	57	.116	rg 59	19.5	10.0	.513	—	—	—
m 11	22.5	17.5	.778	7.00	52	.135	rg 60	20.5	10.5	.513	—	—	—
m 12	20.5	19.0	.927	7.12	59	.121	rg 61	20.5	10.5	.513	—	—	—
m 13	21.0	13.5	.643	6.25	46	.136	eg 62	18.5	10.5	.568	—	—	—
m 14	19.5	19.0	.974	6.88	56	.123	eg 63	19.5	10.5	.538	—	—	—
m 15	26.0	18.0	.692	7.25	56	.129	g 64	23.5	10.5	.447	—	—	—
m 16	23.0	17.0	.739	6.75	57	.118	g 65	16.0	9.5	.594	4.13	33	.125
m 17	21.0	18.0	.857	7.12	55	.129	g 66	19.5	10.0	.513	4.50	44	.102
m 18	21.0	17.0	.810	7.12	55	.129	g 67	17.0	7.0	.412	4.38	41	.107
m 19	20.5	16.0	.781	6.38	56	.114	g 68	16.0	8.0	.500	4.13	37	.112
m 20	22.5	15.5	.689	6.25	49	.128	g 69	18.0	9.0	.500	4.25	42	.101
r 21	20.0	11.5	.575	5.38	45	.120	g 70	18.5	8.5	.459	4.25	41	.104
r 22	21.5	11.0	.512	5.88	48	.123	g 71	19.0	8.5	.447	4.38	41	.107
r 23	18.5	10.0	.541	5.00	44	.114	g 72	16.5	8.5	.515	4.50	37	.122
r 24	20.0	11.0	.550	5.63	48	.117	g 73	18.5	9.5	.514	4.38	44	.100
r 25	19.0	11.0	.579	5.38	44	.122	g 74	17.5	9.5	.543	4.88	43	.113
r 26	20.5	11.0	.537	5.75	49	.117	g 75	19.5	8.0	.410	4.13	43	.096
r 27	19.5	11.0	.564	5.38	50	.108	g 76	18.5	8.0	.432	4.38	40	.110
r 28	19.0	10.5	.553	5.50	45	.122	g 77	19.0	9.0	.474	4.38	40	.110
r 29	21.5	11.0	.512	5.75	50	.115	g 78	20.5	9.0	.439	5.00	44	.114
r 30	20.0	11.5	.575	5.75	46	.125	g 79	17.0	9.5	.559	4.75	44	.108
r 31	21.5	10.0	.465	5.50	43	.128	g 80	18.5	9.0	.486	4.25	42	.101
r 32	20.5	12.0	.585	5.63	47	.120	g 81	16.5	9.0	.545	4.88	43	.113
r 33	20.0	10.5	.525	5.25	49	.107	g 82	17.5	9.0	.514	4.38	45	.097
r 34	21.5	12.5	.581	5.50	47	.117	g 83	19.0	9.0	.474	4.50	47	.096
r 35	17.5	12.0	.686	5.38	43	.125	g 84	18.5	7.5	.405	4.50	45	.100
r 36	21.0	12.5	.595	5.38	47	.114	g 85	18.5	8.5	.459	4.38	46	.095
r 37	21.0	11.5	.548	5.50	47	.117	g 86	17.5	9.0	.514	4.75	41	.116
r 38	21.0	12.0	.571	5.88	49	.120	g 87	18.5	9.5	.514	4.63	45	.103
r 39	19.5	10.5	.538	5.63	46	.122	g 88	16.5	11.0	.667	4.75	46	.103
r 40	19.0	11.0	.579	5 50	48	.115	g 89	23.0	9.0	.391	5.50	51	.108
r 41	18.0	11.5	.639	5.25	43	.122	g 90	22.0	8.0	.364	4.88	48	.102
r 42	21.5	10.5	.488	5.38	48	.112	g 91	18.0	10.0	.556	4.88	49	.100
r 43	23.0	11.0	.478	5.75	50	.115	g 92	21.5	9.0	.419	5.00	48	.104
r 44	22.5	11.5	.511	6.00	51	.118	g 93	18.5	9.0	.486	4.63	47	.099
r 45	19.0	13.0	.684	5.38	46	.117	g 94	20.5	8.0	.390	4.75	46	.103
h 46	20.0	8.5	.425	4.63	44	.105	g 95	21.0	10.0	.476	5.13	47	.109
r 47	22.5	14.0	.622	6.38	49	.130	g 96	21.0	8.0	.381	4.63	47	.099
r 48	21.0	12.5	.595	6.00	48	.125	g 97	22.5	9.5	.422	5.38	50	.108
g 49	17.5	9.0	.514	4.50	43	.105	g 98	18.0	10.0	.556	5.25	54	.097

Abbreviations: Spn. No. = specimen number, which corresponds with number in figures 3–6; T = length of 8th tergite × 8; SU = length of superuncus × 8; SU/T = ratio of superuncus to 8th tergite; V = length of right valva; FW = length of right forewing; V/FW = ratio of valva to forewing. All measurements in mm. m = *Papilio multicaudatus*, r = *P. rutulus*, g = *P. glaucus*, e = *P. eurymedon*, rg = F_1 hybrid of female *P. rutulus* × male *P. glaucus*, eg = F_1 hybrid of female *P. glaucus* × male *P. eurymedon*, h = wild hybrid, see text, a = *P. alexiares*.

TABLE 4—*Continued*

Spn. no.	T	SU	SU/T	V	FW	V/FW
e 99	17.5	11.5	.657	5.50	44	.125
e 100	16.5	10.0	.606	5.13	46	.112
e 101	17.0	11.0	.647	5.13	46	.112
e 102	18.5	11.5	.622	5.13	46	.112
e 103	19.5	10.0	.513	5.25	43	.122
e 104	16.0	11.5	.719	5.25	44	.119
e 105	17.0	10.5	.618	5.13	38	.135
e 106	17.5	10.5	.600	5.25	41	.128
e 107	19.0	10.5	.553	5.50	46	.120
e 108	19.0	12.0	.631	6.13	51	.120
e 109	19.0	11.5	.605	5.63	47	.120
e 110	21.0	11.0	.524	5.87	48	.122
e 111	19.5	10.5	.538	5.25	46	.114
e 112	19.5	10.5	.539	6.00	47	.128
e 113	19.5	9.5	.487	5.63	48	.117
e 114	19.0	10.5	.553	5.38	46	.117
e 115	19.5	12.0	.615	5.75	51	.113
e 116	21.5	11.5	.535	5.87	50	.117
e 117	16.5	11.0	.667	5.75	45	.128
e 118	21.5	13.5	.628	5.63	50	.113
a 119	22.0	10.5	.477	6.00	55	.109
a 120	22.5	12.5	.556	6.13	55	.111
121–126 = *P. troilus* group						
g 127	—	—	—	6.51	61	.107
g 128	—	—	—	6.13	59	.104
g 129	—	—	—	6.38	59	.108
g 130	—	—	—	6.13	61	.100
g 131	—	—	—	6.13	58	.106
g 132	—	—	—	6.25	63	.099
g 133	—	—	—	6.25	61	.102
g 134	—	—	—	6.25	63	.099
g 135	—	—	—	6.00	63	.095

tracing the right valvae, from which India ink drawings were made. After the drawings were completed, each was checked back with the original preparation, and errors were corrected.

The photographs of the eighth tergite, superuncus, and their associated structures were used for direct measurements of the length of the superuncus from tip to base, and the greatest straight-line distance from this base to where the eighth tergite articulates with the seventh. Measurements of the length of the right valvae were also made from the photographs. These were taken from the point where the valva (actually the sacculus) articulates ventrally with the vinculum to the apex of the valva. All measurements, taken to the nearest 0.5 mm, were thus 8 ×. Forewing measurements were taken directly from the butterflies with a pair of calipers. The length of the forewing represents the distance from the base of the wing to its apex, to the nearest mm. The valva length as measured from the 8 × photograph was divided by 8 before the data were tabulated (table 4).

In this study only right valvae were figured. A considerable amount of variation was noted between the right and left valvae of individual specimens, but in general this variation appeared to be no greater than that in a series of right or left valvae from several specimens.

The valvae are drawn so that the dorsal spine complex is to the left and the ventral spine to the right. The curved edge at the top of each figure represents the most posterior part of the valva, and the tubular structure at the base of the valva (sacculus) represents its most anterior part.

1. *P. palamedes, P. pilumnus, P. troilus*

A comparison of the figures of the right valva of *P. pilumnus* and *P. troilus* (figs. 5–6, Nos. 121–124) at once shows that the shape of the valva and the basic form of the armature are strikingly similar in these two species and in contrast to those of all the species in the *P. glaucus* group. Furthermore, they share a common form with *P. palamedes* (Nos. 125–126). On this basis *P. pilumnus* should be grouped with *P. palamedes* and *P. troilus*.

2. *P. alexiares, P. eurymedon, P. glaucus, P. multicaudatus, P. rutulus*

A comparison of the series of valvae of the five species indicates several facts (see figs. 3–6). First, *P. glaucus* is the only species in which the dorsal spine of all individuals is fundamentally bifurcated into two hooks. In some specimens from each area there is a tendency for the development of more than two of these hooks. In contrast to *P. glaucus*, each of the three western species (and *P.*

alexiares) has a dorsal spine which is basically single. Although this spine is strikingly reduced in *P. eurymedon* as compared to all the other species, it is clearly a single structure. Of the three western species, *P. rutulus* is the only one in which a few individuals, e.g., No. 28 from Colorado and No. 35 and No. 36

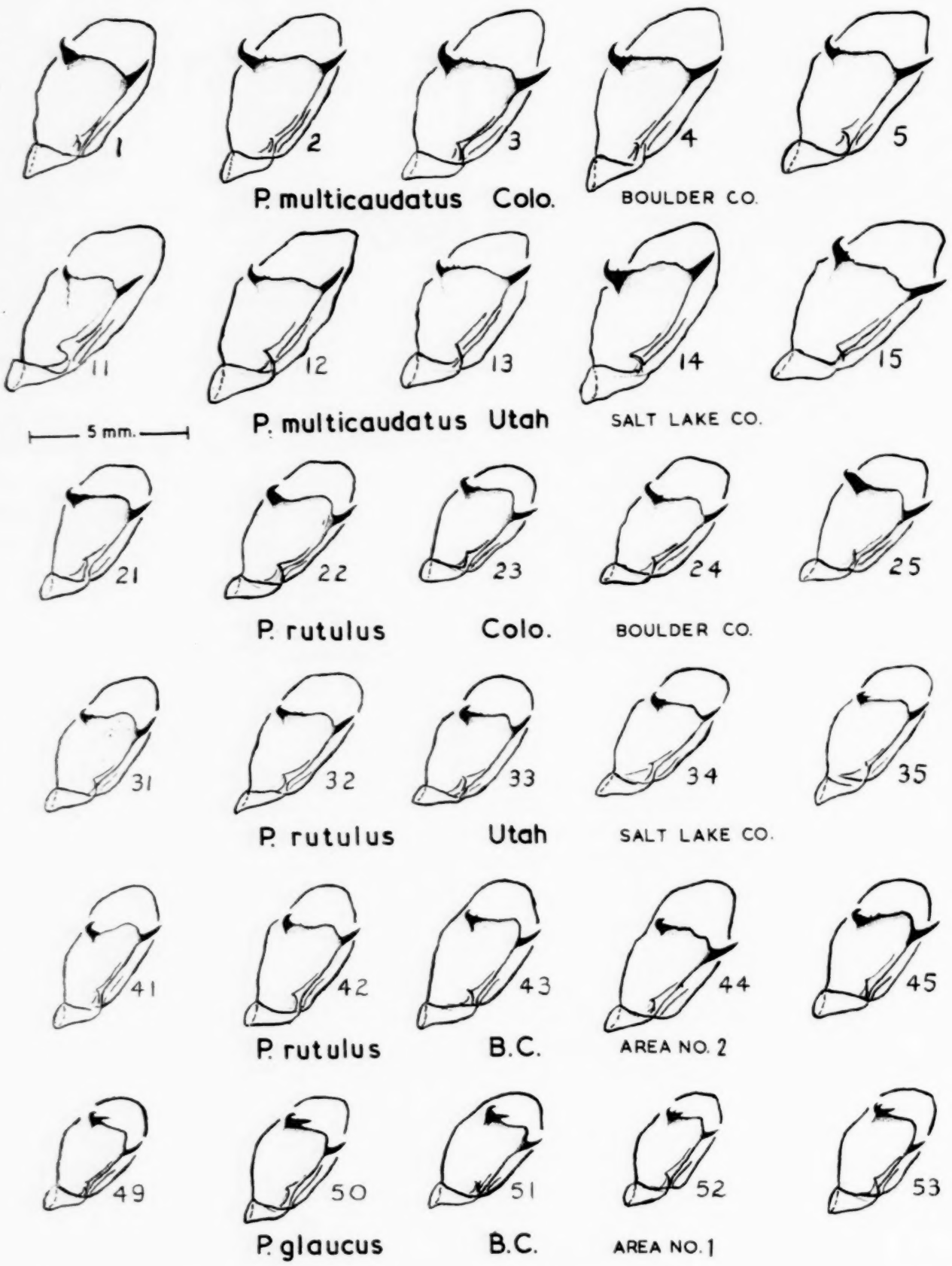

FIG. 3. Right male genital valvae (clasping organs) of *P. glaucus* group from various geographic areas. Exact data are in table 2. Dorsal spine is to left, ventral spine is to right. Each horizontal series is continued in figure 4. For description see text.

from Utah, show a tendency to approach *P. glaucus*. Moreover, *P. rutulus* not only approaches *P. glaucus* at one extreme with respect to this character, but it also intergrades with *P. multicaudatus* (compare No. 47 and No. 8, No. 21 and No. 20). The dorsal spine of *P. rutulus* also seems to grade into those few *P.*

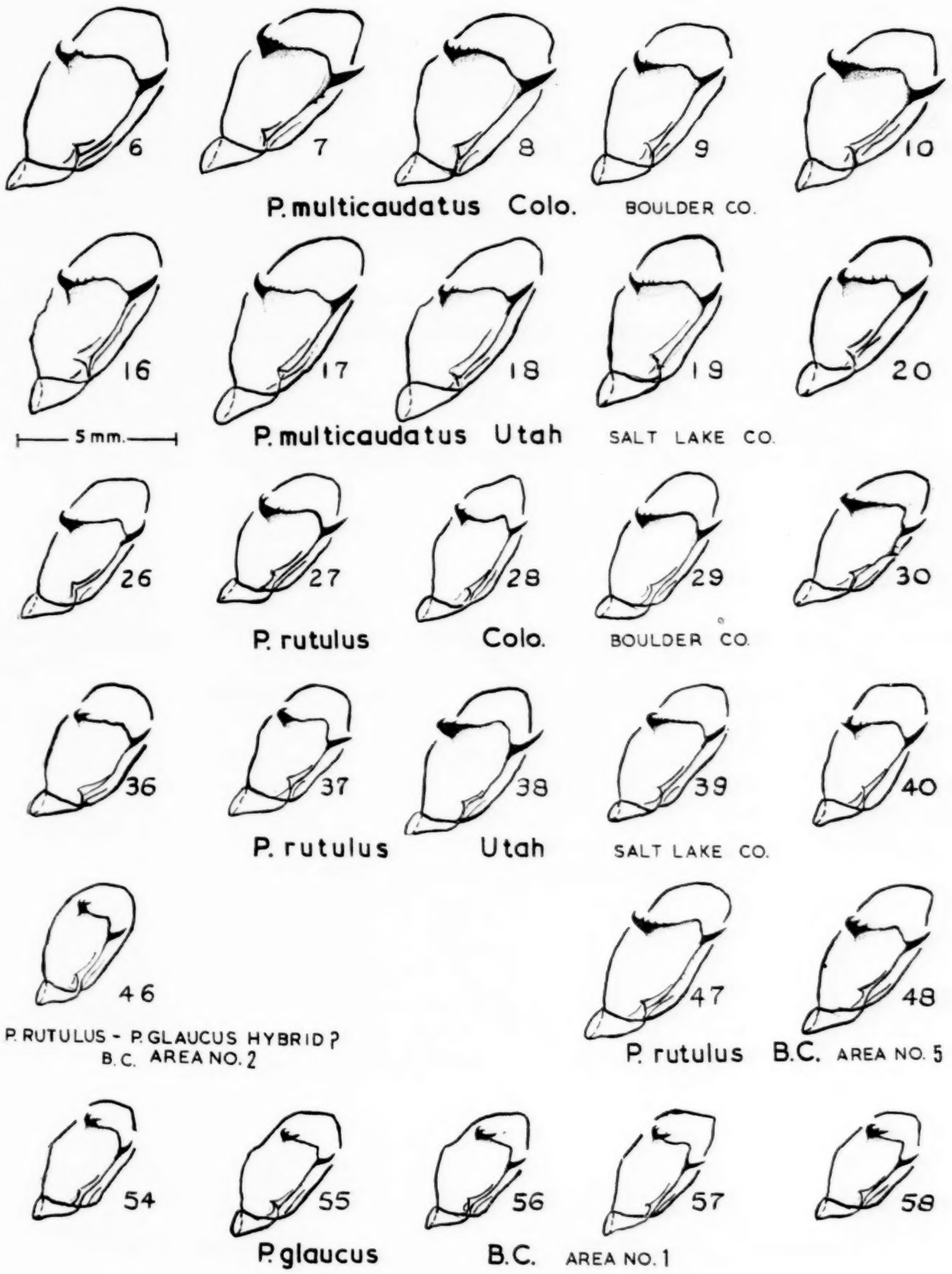

FIG. 4. Right male genital valvae of *P. glaucus* group from various geographic areas. Exact data are in table 2. Dorsal spine is to left, ventral spine is to right. Each horizontal series is continued from figure 3. For description see text.

eurymedon which have some development of this spine (compare No. 32 and No. 108, No. 41 and No. 118). Furthermore, this specimen of *P. rutulus* (No. 41) also approaches *P. alexiares* (No. 119). Thus the form of the dorsal spine in *P. rutulus* is not only distinct from *P. glaucus* when the whole series is con-

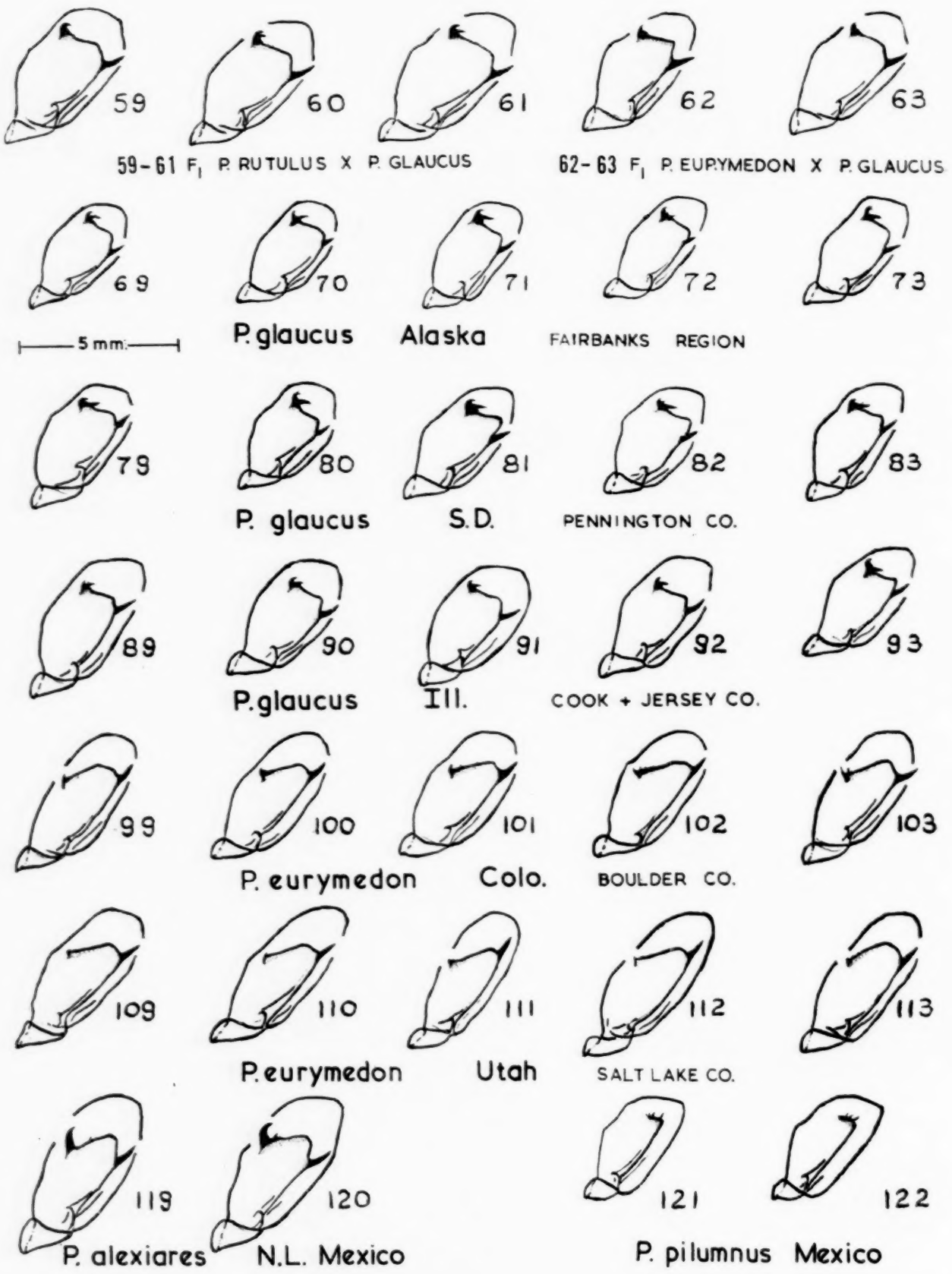

FIG. 5. Right male genital valvae (clasping organs) of *P. glaucus* group and *P. troilus* group from various geographic areas. Exact data are in table 2. Dorsal spine is to left, ventral spine is to right. Each horizontal series is continued in figure 6. For description see text.

sidered, but *P. rutulus* lies at a morphological center and seems to be the most generalized of all the species.

Second, and perhaps less obvious, *P. glaucus* is the only species in which the ventral spine is relatively weak. In most *P. glaucus* from all the areas represented, it is shorter and/or thinner than in the

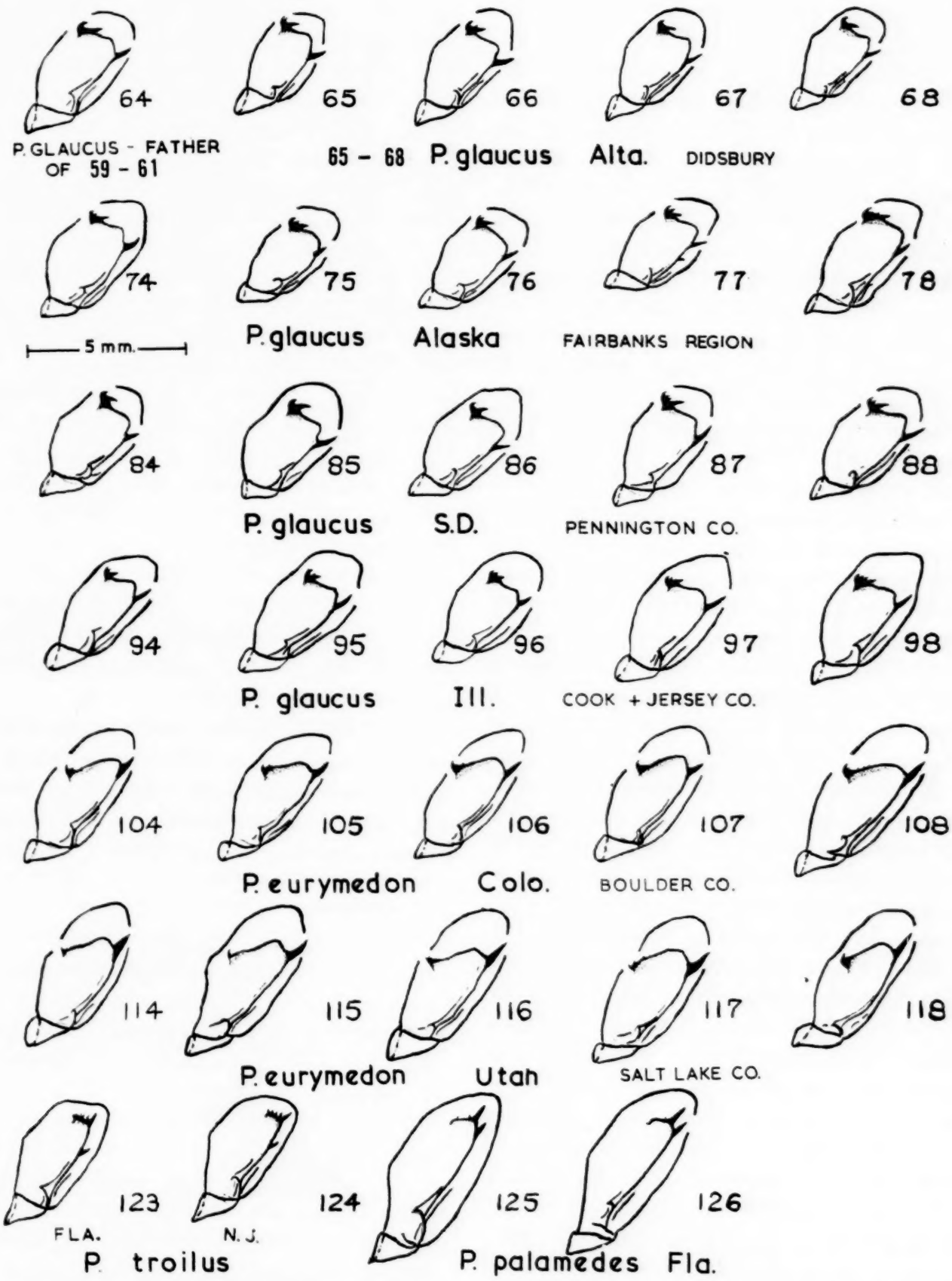

FIG. 6. Right male genital valvae of *P. glaucus* group and *P. troilus* group from various geographic areas. Exact data are in table 2. Dorsal spine is to left, ventral spine is to right. Each horizontal series is continued from figure 5. For description see text.

other species. Exceptions are No. 50 and No. 51 from Cranbrook (British Columbia Area No. 1), No. 81 and No. 85 from the Black Hills of South Dakota, and possibly No. 90 from Illinois. One specimen of *P. rutulus* (No. 28 from Colorado) has this spine weakly developed.

While the valvae of *P. eurymedon* and *P. rutulus* are similar in the appearance of their ventral spines and occasionally in the appearance of their dorsal spines, they are quite different in two other ways. In *P. eurymedon* the valva itself is more attenuated, and the line between the dorsal and ventral spines in the drawings slopes rather steeply to the left. In *P. rutulus,* as in *P. multicaudatus,* this line is more or less horizontal, while in *P. glaucus* it slopes to the right. In this respect *P. alexiares* seems closest to *P. eurymedon.*

A comparison of the three laboratory-reared F_1 hybrids of *P. rutulus* × *P. glaucus* (Nos. 59–61) with the two parental species suggests that they are all like *P. rutulus* with respect to the ventral spine, but the dorsal spine of No. 59 somewhat resembles *P. rutulus* (No. 31), that of No. 60 appears intermediate, and No. 61, like *P. rutulus* No. 36, approaches *P. glaucus.* It is of great interest to note that another somewhat similar intermediate dorsal spine was found in a single Robson specimen (No. 46, British Columbia Area No. 2) which could not be determined as either *P. glaucus* or *P. rutulus* on the basis of submarginal lunule coloration. On the other hand, South Dakota specimens Nos. 80, 81, 82, 83, 86, and 88, could also not be determined from the lunules; yet the dorsal spines of their genitalia are all more similar to *P. glaucus* than to *P. rutulus.* Likewise, the two specimens (No. 53 and No. 54) dissected from Cranbrook (British Columbia Area No. 1) which were placed in the "undetermined" category on the basis of lunule coloration also are similar to *P. glaucus* in their genitalia. The one *P. glaucus*-like specimen from Colorado (No. 22) proved to be *P. rutulus*-like in its genitalia. All other specimens of *P. glaucus* and *P. rutulus* which were dissected were consistent on the basis of both lunule coloration and genitalia. Unfortunately, the seven "undetermined" British Columbia Area No. 3 specimens listed in table 1 were unavailable when the dissections were made. The F_1 hybrids between *P. eurymedon* and *P. glaucus* (Nos. 62–63), which had the same *P. eurymedon* father but different *P. glaucus* mothers, have the ventral spine of *P. eurymedon,* the valva shape of *P. glaucus,* and a dorsal spine which does not resemble either parent. Finally, although no laboratory hybrids are yet available for comparison, it is probable that No. 29 from Colorado is a wild F_1 hybrid of *P. rutulus* × *P. multicaudatus.* This was first suggested to me by the fact that this butterfly is intermediate in its general appearance (width of black stripes, scalloping of hindwings, and ground color), and it can be seen from the figure (No. 29) that all the various valval characters more closely approach one of the *P. multicaudatus* figures than any of the other *P. rutulus* specimens (compare with No. 19).

Observation of the photographs of the superuncus and the eighth tergite of each specimen suggested that the three western species as a whole have a longer superuncus than *P. glaucus.* Figure 7

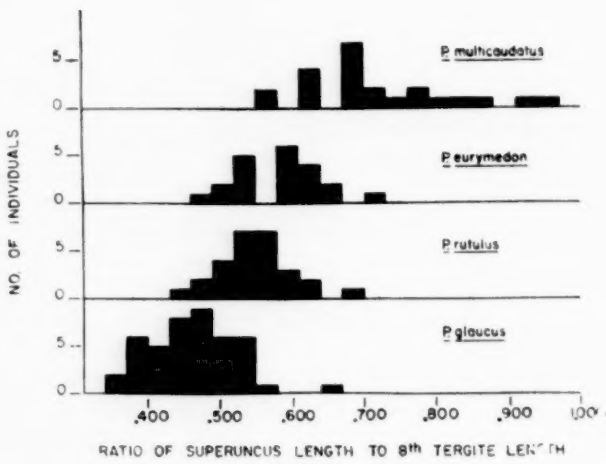

FIG. 7. Histogram showing distribution of values for superuncus/8th tergite ratio in *P. glaucus* group. Exact data are in table 4. For description see text.

(table 4) is a histogram showing the distribution of the values obtained from calculating the ratio of the length of the superuncus to that of the eighth tergite in these four species. The number of specimens of *P. alexiares* available was insufficient for this as well as the next analysis. Because the armature of the valvae of all the "undetermined" specimens which were dissected (with the exception of the one probable hybrid mentioned above) suggested that they were *P. glaucus,* they were regarded as belonging to this species. The mean for the distribution of values in 45 *P. glaucus* specimens is .479. The 95% confidence interval of this mean ranges from .460 to .498. The mean for 27 *P. rutulus* is .563, with a range of .542 to .584; for 20 *P. eurymedon,* .593, with a range of .565 to .621; and for 20 *P. multicaudatus,* .740, with a range of .689 to .791. Thus the relative length of the superuncus is considerably shorter in *P. glaucus* than in the three western species. In these there is substantial overlap of *P. rutulus* and *P. eurymedon,* but in both it is shorter than in *P. multicaudatus.*

In addition to these differences in superuncus length, a comparison of the photographs of the valvae of the four species suggested that the valva length relative to the forewing length is considerably greater in the western species than it is in *P. glaucus.* Figure 8 is a scatter diagram of these measurements (table 4) for the same specimens plotted in figure 7, with one change. This was the addition of a series of nine *P. glaucus* males from the giant Florida population known as *P. glaucus australis* Maynard. The plot immediately suggested two interesting facts. First, the three western species all fall more or less about one line, and differ from *P. glaucus* which falls about another line of less slope. The lines in figure 4 were fitted by the method of least squares. The formula for the estimated line for the *P. glaucus* points is $y = 4.925 + .083\ (x - 46.77)$, and the mean square deviation from the line = .076. For the three western species, $y = 5.924 + .120\ (x - 49.06)$ and the mean square deviation = .102. The mean for the valva/forewing ratio in 53 specimens of *P. glaucus* = .106, and the 95% confidence interval of this mean ranges from .104 to .108. The mean for 27 *P. rutulus* is .119 with a range of .117 to .121; for 20 *P. eurymedon,* .120, with a range of .117 to .123; and for 20 *P. multicaudatus,* .125, with a range of .122 to .128. Thus the three western species are much closer to each other than any one is to *P. glaucus.* The second interesting fact is that *P. rutulus* and *P. eurymedon,* which are the most alike with respect to this valva/forewing ratio, are almost completely overlapping in the absolute size of these two characters. This can be seen in figure 8.

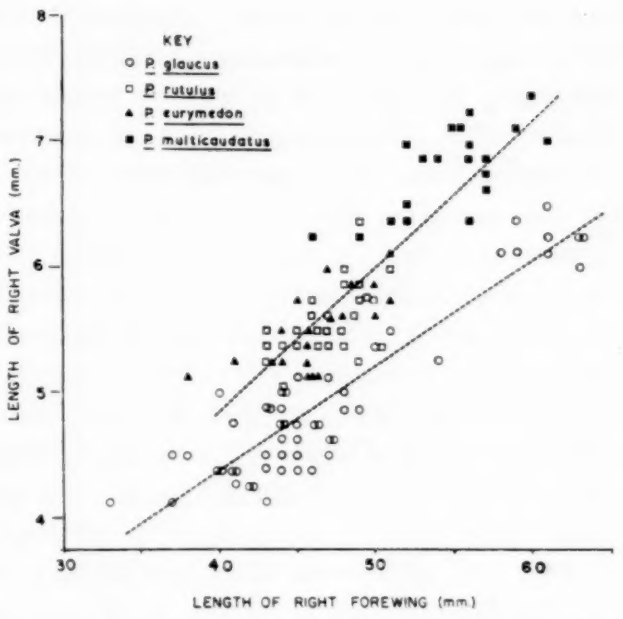

FIG. 8. Scatter diagram for length of right valva plotted against length of right forewing in *P. glaucus* group. Note that *P. glaucus* falls about one line and the three western species about another. Exact data are in table 4.

DISCUSSION AND CONCLUSIONS

1. Removal of *P. pilumnus* from the *P. glaucus* Group

The general appearance of the adult of *P. pilumnus* is so like the members of the *P. glaucus* group that Edwards (1897) at first found Schaus' (1884) description of its larva and pupa incredible. He thought

that Schaus had probably described the early stages of *P. palamedes,* and said that "certainly the pupa of *pilumnus* would be of the same character as that of *daunus* (= *multicaudatus*), *rutulus* and *turnus* (= *glaucus*)." Even though in a supplementary note (1897) Edwards acknowledged that Schaus had been correct, "so discovering that *pilumnus* belongs to the *troilus* and *palamedes* group instead of that of *turnus*" (= *glaucus*), no other authors except Forbes (1951) have suggested placing it with the *P. troilus* group. There are three main factors which suggest that *P. pilumnus* does not belong to the *P. glaucus* group. First, as shown in this paper, the valvae of the male genitalia in two specimens each of *P. pilumnus, P. troilus,* and *P. palamedes* resemble each other more closely both in shape and in the general plan of the armature than any of them resembles any of the *P. glaucus* group specimens. Second, a comparison of Schaus' original description of the mature larva of *P. pilumnus* with the same stage of *P. troilus* and *P. palamedes* on the one hand and members of the *P. glaucus* group on the other clearly indicates that *P. pilumnus* is much more like the former two species in several pattern characters. Finally, his description of both the colors and morphology of the pupa also places it with *P. troilus* and *P. palamedes.* Thus the *P. glaucus* group should be reduced from six to five species: *P. alexiares, P. eurymedon, P. glaucus, P. multicaudatus,* and *P. rutulus.*

2. Morphological Relationships within the *P. glaucus* Group

Even though *P. glaucus* and *P. rutulus* are very similar in the appearance of their adults, a study of several aspects of the morphology of all the species in the group has indicated that *P. rutulus* is closer to *P. eurymedon* and *P. multicaudatus* than it is to *P. glaucus.* The mature larvae of *P. rutulus* have an eye-spot character composed of a double element which is also found in both *P. multicaudatus* and *P. eurymedon,* but is absent in *P. glaucus. P. rutulus* was again found to be allied with *P. eurymedon* and *P. multicaudatus* in that most specimens possessed a single-hooked dorsal spine as compared to the double-hooked dorsal spine of *P. glaucus.* It likewise shared a common plan with these in having a heavy ventral spine of the valval armature while that of *P. glaucus* is comparatively weak. Furthermore, the ratio of the valva length to the wing length was quite similar in these three and contrasted with *P. glaucus* in which it was considerably smaller.

P. eurymedon and *P. rutulus* appear to be more closely related than either is to *P. multicaudatus.* This is suggested both by the eye-spot pattern of the mature larvae and by measurements of various parts of the genitalia. For example, the ratio of the length of the superuncus to the length of the eighth tergite is quite similar in these two, but much larger in *P. multicaudatus.* Moreover, the absolute length of the valvae and forewings are nearly identical in these and both are considerably smaller than in *P. multicaudatus.* On the other hand, the attenuation of the valva, the reduction of the dorsal spine, the greater "slope" of the line between the dorsal and ventral spines, and the whitish ground coloration of the wings all differentiate *P. eurymedon* rather strikingly from *P. rutulus.* Elsewhere I have presented evidence that the whitish coloration of *P. eurymedon* females is a barrier to interspecific courtship by males of the yellow *P. multicaudatus* (Brower, 1959). It is possible that the obvious genital differences as well as the whitish coloration in *P. eurymedon* have all been intensified by selection because of their value as partial barriers to hybridization with the otherwise exceedingly similar *P. rutulus.*

Although *P. rutulus* is morphologically closer to *P. eurymedon* and *P. multicaudatus* with which it is widely sympatric, it appears to lie between them and *P. glaucus* in the superuncus/eighth tergite ratio and the valva/forewing ratio. More-

over, it is also unique with respect to the dorsal spine of the valva because it is the only species in which specimens occur that intergrade with all the members of the group. For these reasons, *P. rutulus* appears to be at the morphological center of the *P. glaucus* group.

The hybridization experiments carried out in the laboratory by Clarke and Sheppard (1955, 1958) have suggested that there is some genetic incompatability produced in the hybrids of *P. glaucus* × *P. rutulus* which is even greater in the hybrids of *P. glaucus* × *P. eurymedon*. These authors found that the F_1 hybrids of a *P. rutulus* female from Washington crossed with a *P. glaucus* male from Illinois exhibited a considerable degree of infertility and that crosses of Illinois *P. glaucus* females × California *P. eurymedon* males produced pupae more than half of which gave rise to only males the following spring. The rest of the pupae are overwintering a second year and from their large size appear to be females (the heterogametic sex in Lepidoptera). These results are important because they substantiate the conclusion which was arrived at one the basis of comparative morphology, namely that *P. rutulus* lies between *P. eurymedon* and *P. glaucus*, and they also support the view that *P. glaucus* and *P. rutulus* are separate species.

3. Marginal Overlapping and Hybridization in *P. glaucus* and *P. rutulus*

A study of the geographic variation of orange coloration in the submarginal lunules on the underside of the hindwings in museum specimens of *P. glaucus* and *P. rutulus* has shown that *P. rutulus*, along with *P. eurymedon* and *P. multicaudatus*, replaces *P. glaucus* in western North America, but narrow overlapping does occur at the extremities of their ranges. The region of overlap for which the most data are available is British Columbia and it is notable that several cases of overlapping distribution of bird species and subspecies have previously been reported from this area. Rand (1948) has summarized these, and is of the opinion that they represent examples of Pleistocene disjunction of once continuous populations into refugia in the southwest and southeast, followed by reexpansion and contact after the retreat of the ice. This explanation seems quite plausible in the case of these four butterflies, where *P. eurymedon, P. multicaudatus,* and *P. rutulus* are more similar to each other in several important morphological characters than any one is to *P. glaucus*. Presumably, they have each originated from a southwestern isolate and *P. glaucus* from a southeastern one, but as Deevey (1949) has pointed out, present distributions are no sure indication of those in the past.

The data presented in this paper suggest that natural hybridization takes place between populations of *P. glaucus* and *P. rutulus* in the zone of overlap. However, in two areas where both are common relative to each other, the number of hybrids is greatly reduced from the number expected on the assumption of random mating of the parental species and normal viability of the hybrids. These two butterflies should therefore be regarded as separate species. Dobzhansky's (1937) hypothesis that natural selection has improved barriers to hybridization because of reduced success of the hybrids in comparison to the purebreds is put forth to explain the hybrid deficiency. This hypothesis is supported by the finding that the expected number of hybrids *is* produced in a third area in British Columbia. Here only one species predominates and the other is rare. The implication of this is that natural selection has had less overall opportunity to operate against the formation of hybrids where only one species is common, because the proportion of hybrids produced would be fewer than in areas where both species are common relative to each other. Direct evidence in favor of Dobzhansky's hypothesis has been obtained in the laboratory. In 1938 Dobzhansky and Koller reported that in-

dividuals of both *Drosophila pseudoobscura* and *D. miranda* from geographically adjacent populations are less prone to mate than are individuals from distant populations. However, Spieth (1951) found no such correlation when he observed the mating behavior of interstrain crosses of species of the *D. virilis* group, and he concluded that the degree of development of sexual isolation is randomly distributed; this supports Muller's (1940) hypothesis that sexual isolating mechanisms arise as a by-product of genetic divergence because of local adaptations in geographically separate populations. Koopman (1950) showed that the frequency of mating between *D. pseudoobscura* and *D. persimilis* could be reduced by eliminating all of the hybrids formed in several successive generations, and Knight, Robertson, and Waddington (1956) have shown that the frequency of mating between two mutant stocks within a single species can be reduced by similar anti-hybrid selection. It is thus apparent that selection is able to enhance sexual isolating mechanisms, but whether or not it does in nature appears to depend on the particular organisms or even the particular populations under study. Blair (1955a,b) has provided indirect evidence that such selection has taken place in two North American toads of the genus *Microhyla* which are known to hybridize to a limited extent. These have very similar mating calls where they are allopatric, but where they overlap in eastern Texas and Oklahoma there is a striking difference between their calls. Moreover, there is a pronounced difference in size in the overlap area as compared to distant populations, and Blair thinks that this may be important in reducing the willingness of males to clasp females of each other's species. It is interesting to note that much of the evidence that selection has reduced food competition between closely related species in nature is also indirect, as for example in the accentuated beak differences in areas of overlap which have been found in Darwin's Finches (Lack, 1947), the Hawaiian honeycreepers (Amadon, 1950a), and the Middle Eastern rock nuthatches (Vaurie, 1951).

In addition to the butterflies discussed in this paper, other animals in which hybridization occurs to varying extents in different places have been reported, and as Amadon (1950b) has pointed out, "since the attainment of sexual isolation . . . is a gradual process, we can not expect that it will occur simultaneously throughout populations that come in contact over a long distance or in several different areas." Sibley's (1954) study of the spotted and collared towhees of Mexico (*Pipilo ocai* and *P. erythrophthalmus*) is such an example. These two birds are morphologically distinct and in at least one locality are sympatric with no evidence of interbreeding, while at other points of contact on the Mexican plateau varying degrees of hybridization appear to be found, including complete clinal intergradation. Free interbreeding in some areas and its absence in others probably also occur in *Pieris* butterflies of Europe. In the northern Alps *P. napi* predominates in the valleys, with *P. bryoniae* higher in the mountains, and although there is some spatial and temporal overlapping, hybridization is either absent or rare. In the mountains of Scandinavia a *P. bryoniae*-like butterfly occurs which is called *P. napi adalwinda*. South of these mountains *P. napi napi* is found, and a nearly smooth cline exists between it and the northern mountain form (Petersen, 1952). However, in the southern and eastern Alps where *P. bryoniae* has more than one brood per year, varying degrees of hybridization and introgression seem to occur (Petersen, 1955). Brown and Wilson (1956) have reviewed a situation found in the Australian honeyeaters of the genus *Myzantha* which is similar to that found in the Scandinavian butterflies. To the southeast in the Victorian mallee district the light colored *M. f. flavigula* nests together with the darker *M. melanotis* and there is no hybridization. From here

through the arid south-central region *M. f. flavigula* extends westwards and intergrades along a cline with the southwestern *M. f. obscura* which is morphologically very much like the eastern *M. melanotis*. Thus *M. f. flavigula* acts as a biological species with the dark eastern representative, but intergrades completely with the similar western one.

It is possible that the adult color criterion on which the *P. glaucus* × *P. rutulus* hybrids have been determined is not completely correct and it will be necessary for further investigation to confirm these data. A source of error could also result from the fact that some specimens from the center of the range of *P. rutulus* have orange in their lunules and therefore are *P. glaucus*-like. These rare specimens may represent variants produced through introgression from *P. eurymedon* or *P. multicaudatus*, both of which often have much orange in their corresponding lunules. In fact, one male specimen from Colorado is almost certainly a hybrid of *P. rutulus* × *P. multicaudatus*.

In contrast to the three areas in British Columbia where the observed number of hybrids is less than or equal to the expected, there is a highly significant excess of them in the Black Hills of South Dakota and also in B. C. Area No. 1. That this may be due to the introgression of characters from the rarer *P. rutulus* into the *P. glaucus* populations is suggested by the fact that none of these hybrid-like specimens which were examined approaches the laboratory-reared F_1 hybrids with respect to the dorsal spine of their genitalia.

In conclusion it appears that *P. glaucus* and *P. rutulus* are representatives of two previously isolated populations of a former single species which have come to overlap each other and are now at a stage in the speciation process where sexual isolating mechanisms have evolved in some areas but not in others. The future study of these two butterflies in British Columbia in conjunction with laboratory experiments to determine the degree of hybrid inferiority should provide an outstanding opportunity to test the hypothesis that isolating mechanisms can be favored by selection in cases of secondary overlap in nature.

Acknowledgments

I should like to express my appreciation to Dr. C. L. Remington of Yale University for originally suggesting this problem and for encouragement and help throughout its development. Special thanks are also due Professor G. E. Hutchinson, Drs. W. D. Hartman, and P. F. Bellinger of Yale University for reading the manuscript and offering many helpful suggestions. Dr. E. B. Ford, F.R.S. and Dr. A. J. Cain of Oxford University have offered stimulating discussion, as has Dr. P. M. Sheppard of the University of Liverpool who also kindly supplied the laboratory-reared hybrids. Drs. E. G. Munroe and T. N. Freeman of the Canadian Department of Agriculture provided generous assistance while I was gathering records from the very fine Canadian National Collection. Dr. A. Petrunkevitch gave me much useful advice on microphotography. I am also grateful to Dr. R. L. Chermock of the University of Alabama and Dr. J. F. G. Clarke and Mr. W. D. Field of the United States National Museum, to Dr. F. H. Rindge of the American Museum of Natural History, to Messrs. C. F. dos Passos of Mendham, New Jersey, D. Eff of Boulder, Colorado, and W. L. Phillips of Salt Lake City, Utah, each of whom has assisted me in gathering data and specimens. My friend, Mr. J. G. Coutsis of Athens, Greece, deserves a note of special thanks for his help with the drawings. My wife, Dr. Jane Van Zandt Brower, has been of inestimable help throughout the course of my work.

This paper represents one aspect of my Yale University Ph.D. dissertation which has been supported by two National Science Foundation Predoctoral Fellowships and in part by a Sigma Xi RESA Grant.

Summary

1) This paper is a study of morphological interrelationships and natural hybridization in several closely related North American butterflies belonging to the *Papilio glaucus* group.

2) On the basis of the color pattern of the mature larvae, pupal morphology, and male genital characters, *P. pilumnus* is removed from the *P. glaucus* group and placed in the *P. troilus* group. This reduces the *P. glaucus* group to the following five species: *P. alexiares, P. eurymedon, P. glaucus, P. multicaudatus,* and *P. rutulus.*

3) A limited study of the thoracic eyespot markings of mature larvae (except *P. alexiares* in which the larva is unknown) indicates that the three species which occur together over extensive areas in western North America (*P. eurymedon, P. multicaudatus,* and *P. rutulus*) are more like each other than any one of them is like the eastern and northern *P. glaucus.*

4) Visual and morphometric comparisons of male genitalia from series of each species also indicate a close alliance of the three western species as opposed to *P. glaucus.* The sample of the Mexican *P. alexiares* was small but suggests that it is closest to the western species.

5) *P. rutulus* is unique in having male specimens with a dorsal armature of the valva which intergrades towards each of the four other species. On this basis, as well as in the superuncus/eighth tergite ratio and valva/forewing ratio, it appears morphologically to be at the center of the whole *P. glaucus* group.

6) *P. rutulus* and *P. eurymedon* are very similar with respect to the thoracic markings of the mature larva, absolute size of the adult, and some characters of the male genitalia. But they are also very different in other characters of the male genitalia as well as in the ground coloration of the wings, which is yellow in *P. rutulus* and whitish in *P. eurymedon.* It is suggested that these differences may have been selected to improve sexual isolation between the two butterflies.

7) A study of the orange coloration in the submarginal lunules on the underside of the hindwings in museum specimens has shown that there is a very abrupt geographic replacement of *P. glaucus* by *P. rutulus* (as well as *P. eurymedon* and *P. multicaudatus*) from north to south and east to west in southern British Columbia, but all four species do overlap in this area. It is suggested that the three western butterflies have descended from a southwestern Pleistocene isolate and *P. glaucus* from a southeastern one and that their present overlapping is the result of range expansion after the retreat of the glacial barriers.

8) In this zone of overlap, specimens occur which may be hybrids between *P. glaucus* and *P. rutulus.* In two of three areas there is a deficiency of these from the number expected on the assumptions of random mating of the parental species and normal viability of the hybrids. The two butterflies should therefore be regarded as separate species. In both of these areas the two are common relative to each other, but in a third where one is rare and the other common, the expected number of hybrids was found. This difference may reflect the fact that natural selection has had less overall opportunity to operate against the formation of hybrids where one species is rare because in such an area fewer hybrids would be produced.

9) In contrast to these three areas in southern British Columbia, there is a highly significant excess of hybrid-like specimens in the Black Hills of South Dakota and in another area in B. C. which may be due to the introgression of characters from the rare *P. rutulus* into the *P. glaucus* populations.

10) A probable hybrid between *P. rutulus* and *P. multicaudatus* from Colorado is noted.

Literature Cited

Amadon, D. 1950a. The Hawaiian Honeycreepers (Aves, Drepaniidae). Bulletin of the American Museum of Natural History, **95**: 151–262.

——. 1950b. The species—then and now. Auk, **67**: 492–498.

Barnes, W., and J. McDunnough. 1916. Notes on North American diurnal Lepidoptera. Contrib. Nat. Hist. Lepid. N. Am., **3**: 53–54.

Blair, W. F. 1955a. Mating call and stage of speciation in the *Microhyla olivacea-M. carolinensis* complex. Evolution, **9**: 469–480.

——. 1955b. Size difference as a possible isolation mechanism in *Microhyla*. American Naturalist, **89**: 297–301.

Brown, W. L., Jr., and E. O. Wilson. 1956. Character displacement. Systematic Zoology, **5**: 49–64.

Brower, L. P. 1959. Speciation in butterflies of the *Papilio glaucus* group. II. Ecological relationships and interspecific sexual behavior. Evolution (in press).

Clarke, C. A., and P. M. Sheppard. 1955. The breeding in captivity of the hybrid *Papilio rutulus* female × *Papilio glaucus* male. Lepidopterists' News, **9**: 46–48.

——, and ——. 1957. The breeding in captivity of the hybrid *Papilio glaucus* female × *Papilio eurymedon* male. Lepidopterists' News, **11**: 201–205.

Deevey, E. S., Jr. 1949. Biogeography of the Pleistocene. Bulletin of the Geological Society of America, **60**: 1315–1416.

Dice, L. R. 1943. The biotic provinces of North America. University of Michigan Press, Ann Arbor.

Dixon, W. J., and F. J. Massey, Jr. 1957. Introduction to statistical analysis. Second edition. McGraw-Hill Book Co., Inc., New York.

Dobzhansky, Th. 1937. Genetics and the origin of species. First edition. Columbia University Press, New York.

——, and P. C. Koller. 1938. An experimental study of sexual isolation in *Drosophila*. Biologischen Zentralblatt, **58**: 589–607.

Edwards, H. 1874. On the transformation of the diurnal Lepidoptera of California and the adjacent districts. Proceedings of the California Academy of Sciences, **5**: 161–172, 325–332.

Edwards, W. H. 1897. The butterflies of North America. Third series, and supplementary notes. Houghton, Mifflin, and Co., Boston.

Forbes, W. T. M. 1951. Footnote on *Papilio*. Lepidopterists' News, **5**: 16.

Knight, G. R., A. Robertson, and C. H. Waddington. 1956. Selection for sexual isolation within a species. Evolution, **10**: 14–22.

Koopman, K. F. 1950. Natural selection for reproductive isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Evolution, **4**: 135–148.

Lack, D. 1947. Darwin's finishes. Cambridge University Press, Cambridge.

Mosher, Edna. 1916. A classification of the Lepidoptera based on characters of the pupa. Bulletin of the Illinois State Laboratory of Natural History, **12**: 17–159.

Muller, H. J. 1940. Bearings of the '*Drosophila*' work on systematics. In the New Systematics, edited by J. Huxley. Oxford University Press, Oxford.

Petersen, B. 1952. The relation between *Pieris napi* L. and *P. bryoniae* Ochs. Transactions of the IXth International Congress of Entomology, **1**: 83–87.

——. 1955. Geographische Variation von *Pieris* (*napi*) *bryoniae* durch Bastardierung mit *Pieris napi*. Zoologiska Bidrag Fran Uppsala, **30**: 355–397.

Rand, A. L. 1948. Glaciation, an isolating factor in speciation. Evolution, **2**: 314–321.

Rothschild, W., and K. Jordan. 1906. A revision of the American *Papilios*. Novitates Zoologicae, **13**: 581–599.

Ruelas, A. 1925. *Papilio daunus* Boisduval. Boletin Oficial de la Secretaria de Agricultura y Fomento, **9**: 144–155.

Schaus, W., Jr. 1884. Early stages of Mexican Lepidoptera. Papilio, **4**: 100–103.

Scudder, S. H. 1889. The butterflies of the eastern United States and Canada. 3 volumes. Published by the author, Cambridge, Massachusetts.

Seurat, L. G. 1898. Metamorphoses du *Papilio daunus*. Memorias de la Sociedad Cientifica "Antonio Alzate," **11**: 34–36.

Sibley, C. G. 1954. Hybridization in the red-eyed towhees of Mexico. Evolution, **8**: 252–290.

Spieth, H. T. 1951. Mating behavior and sexual isolation in the *Drosophila virilis* species group. Behavior, **3**: 105–145.

Vaurie, C. 1951. Adaptive differences between two sympatric species of nuthatches (*Sitta*). Proceedings of the Xth International Ornithological Congress, Uppsala 1950: 163–166.

A STUDY OF HYBRIDS IN *COLIAS* (LEPIDOPTERA, PIERIDAE)[1]

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Received April 13, 1958

The genus *Colias,* of which 57 species were listed by Talbot (1935), is distributed widely in the world. These species resemble one another very closely. Some species have one generation a year and hibernate as larvae, while others have two to several generations a year. The diversity of the food plants is large. Some presumed wild hybrids have been described and discussed. Many species of this genus have been reared, and the genetics of some characters have been investigated (Remington, 1954). The sex-limited "alba" form in *Colias eurytheme, C. philodice,* and *C. erate poliographus* Motschulsky, and interspecific characters between *C. eurytheme* and *C. philodice* have been most extensively studied.

Recently many studies of interspecific hybridization in plants have been carried out to clarify the genetics of interspecific characters and of speciation among closely related species. Some natural or long-cultivated allopolyploid plants have even been synthesized in the laboratory by interspecific crossings. However, the published works upon animals are relatively few. This may be due largely to the difficulty of obtaining interspecific matings among animals, where behavior is often part of pre-reproductive recognition. The genus *Colias* is a good group for study among animals, since presumed natural hybrids suggest the possibility of producing laboratory hybrids, and parallel studies of laboratory and natural hybrids may be expected to prove significant for amplification of evolutionary theory. The wide distribution of this genus and the large number of species may also make possible large scale research in the field of genetics and evolution.

Attempts at interspecific hybridization among North American *Colias* have been carried out by the writer in order to find out if interspecific hybridization actually occurs in nature between various North American species, and, if so, to find out how extensive it is and what are its consequences. The conditions were kept as natural as possible for this purpose. The genetics of interspecific characters were studied by rearing of progeny from successful interspecific matings and from subsequent crosses. Since the physiology and ecology of this genus are still insufficiently known, data on developmental rates, hibernation, food plants, and seasonal isolation were also obtained during the above experiments to clarify the relation between the species of the genus and also to compare the characters of hybrids with those of the parental species. These data were published separately (Ae, in press). One presumed wild hybrid was also studied.

MATERIALS AND METHODS

The species used in this work were: *C. alexandra* Edwards of Gothic, Colorado; *C. eurytheme* Boisduval of Notre Dame, Indiana, and New Haven, Connecticut; *C. interior* Scudder of Cheboygan, Michigan, and Saddle-back Mountain, Maine; *C. meadii* Edwards of Gothic; *C. philodice* Latreille of Notre Dame, New Haven, and Gothic; and *C. scudderi*

[1] Most of the work reported here was presented as a part of a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Notre Dame.

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Reakirt of Gothic. While *C. alexandra, C. interior, C. meadii,* and *C. scudderi* have one generation a year, *C. eurytheme* and *C. philodice* have two to several generations a year. Hibernation of larvae of some of these species may be a true diapause. However, since no clear evidence of this has yet been obtained, the inclusive term hibernation will be used in this paper. Larvae of *C. alexandra, C. eurytheme, C. meadii,* and *C. philodice* feed on legumes such as clovers, alfalfa, and vetch. *C. interior* feeds on blueberry and *C. scudderi* feeds on willows. However, the survey of the food plants of these species has not been completed yet. Their wing patterns resemble one another very closely, and in the range of variation of several characters, they often overlap each other. Therefore, although typical specimens are readily classified by combinations of alar characters such as ground coloration, black borders, and color of fringes and hind wing discal spot, unplaceable specimens occur.

Orange color grade samples of the ground color of the wings, with 9 the reddest and 0 the yellowest, were sent by Dr. W. Hovanitz and used for determination of orange color grades of various hybrids as well as species. Color grades of 10–1 (Hovanitz, 1944a) were replaced recently with 9–0 by that author (correspondence). Color grades of typical *C. eurytheme* are 9 and 8, although reduction into 7 or even lower grades seems to occur as an environmental effect. The color grade of *C. philodice* is usually 0. Color grades of hybrids between the above two species are intermediate, probably ranging from 7 to 1 (Hovanitz, 1944a, 1949). In these standards, the color grades of *C. alexandra, C. interior,* and *C. scudderi* are usually 0; and *C. meadii* has grade 9 or more.

All inter- and intraspecific matings were obtained in cages under either direct sunlight or fluorescent light. The main attempts at interspecific matings were carried out at the actual place and time of flight of the paternal species. Two kinds of cages were used for matings. Type A is cubic, about 28 cm on each side, covered with glass on one side, the other sides with screen, and top and bottom with wood. Type B is also cubic, about 28 cm on each side, covered on all four sides as well as top and bottom with screen. Usually 6 to 12 males, more than a day old in the case of laboratory males, were placed in a cage with 6 to 12 newly emerged females. Copulation, if it occurs at all, usually takes place within one hour, although it may sometimes occur much later. The fluorescent light was set in a windowless constant temperature (80° F) room, and the strength of the light was sufficient to keep vetch seedlings green (about 5000 foot candles) with 14–15 hours illumination a day. The method of hand-pairing has recently become a very convenient tool of interspecific matings among some Lepidoptera (Clarke & Sheppard, 1956). However, in spite of extensive trials, the writer was not successful in applying this method to the genus *Colias.*

Eggs were obtained from females in cages containing the food plants or in glass lamp chimneys on potted food plants. These containers were placed under the sun or near an incandescent lamp.

For the rearing of larvae, insect cages about 30 cm cubed were used. Potted seedlings or older plants were usually used as larval foods. The main food plants used were white clover, alfalfa, vetch, blueberry, and weeping and pussy willows. Larvae were reared in the greenhouse of the University of Notre Dame, and in rearing rooms of the Department of Biology, University of Notre Dame; the Josiah Willard Gibbs Research Laboratory at Yale University; and the Johnson Laboratory of the Rocky Mountain Biological Laboratory. The rearing room of the University of Notre Dame was kept at 80° F, 65% R.H., and 15 hours fluorescent light illumination a day, except when stated to be otherwise, below. The rearing room of the Gibbs

Laboratory was kept at 80° F, and 14 hours fluorescent light illumination a day. The conditions in the other were not controlled.

During the rearing, a large number of larvae and pupae often died of diseases. Larvae usually became soft, brownish-black, decaying masses, after their color had changed to lighter green. Diseased pupae usually became black and dried up if they were left untouched. These symptoms coincide precisely with the description of Steinhaus (1949) concerning polyhedroses by virus infection in *Colias*. Therefore, these losses are referred to simply as virus disease in this paper, although microscopic tests were not performed. Other diseases caused by fungi and bacteria seemed to be negligible.

A mixture of 1 part of clover honey and 2 parts of water was used as food for the adults.

An individual designation was given to each wild female which became the mother of a brood, and to all laboratory reared males and females. The prefix used for *C. eurytheme* and *C. philodice* was W in 1954 and 1955, P in 1956, and E in 1957. The prefixes for *C. alexandra, C. interior, C. meadii,* and *C. scudderi* are, respectively, A, I, M, and S. The individual designation of a female was also used as the designation of its brood. The designation of a brood was used as the prefix of individuals in the brood. For instance, the wild *C. eurytheme* female, W-15, produced the Brood W-15, and one of the females of this brood, W-15-62, mated with a *C. interior* male and produced the Brood W-15-62.

New Data

1. Hybridization Studies

The writer obtained readily many intraspecific matings of *C. eurytheme* and *C. philodice* with the above mentioned method. However, interspecific matings occurred only rarely. The main attempts which were made to obtain interspecific matings are as follows (a—year, b—place, c—crossing):

1. a. 1954 & 1955
   b. The Biological Station (near Cheboygan), University of Michigan
   c. Between females of *C. eurytheme* and *C. philodice* from Notre Dame and local wild males of *C. interior*
2. a. 1956 & 1957
   b. The Rocky Mountain Biological Laboratory
   c. Between females of *C. eurytheme* and *C. philodice* from Notre Dame (1956) and from New Haven (1957) and local wild males of *C. alexandra, C. meadii,* and *C. scudderi*
3. a. 1955
   b. The University of Notre Dame
   c. Between a Maine female of *C. interior* reared at Notre Dame and local males of *C. eurytheme* and *C. philodice*
4. a. 1956
   b. Yale University
   c. Between Colorado females of *C. meadii* reared at Yale University and local males of *C. eurytheme* and *C. philodice*
5. a. 1956
   b. Yale University
   c. Between local *C. eurytheme* and *C. philodice*

The detailed data on these and the matings of their progeny are reported below.

A. *C. Eurytheme* and *C. Philodice* × *C. Interior*

About 500 pupae of *C. eurytheme* and *C. philodice* were obtained from larvae reared on white clover in the greenhouse at Notre Dame. These pupae were brought to the Biological Station, University of Michigan, on 28 June 1954. Many females emerged between 29 June and 9 July.

Matings were tried at Mud Lake and at Elliot's Creek, where *C. interior* is usually found, and outdoors at the Biological Station, using females of *C. eurytheme* which emerged at the laboratory and wild *C. interior* males which were collected at Mud Lake or Elliot's Creek in the vicinity of the Station. In the type B cages, as in the field, *C. interior* males were active only under the bright sun. About 70 males of *C. interior* were used for matings. Only at Elliot's Creek, where two bright afternoons were spent

in the effort, did *C. interior* males readily try to copulate with *C. eurytheme* females. On the second afternoon at the Creek (9 July), one *C. interior* male, caught several minutes before, copulated from 2:08 to 3:15 P.M. with an orange *C. eurytheme* female which had emerged within 30 minutes.

Mating outdoors at the Station was tried, with mating between *C. eurytheme* females and males as a control. Although a total of 13 matings were obtained readily between *C. eurytheme* females and males, no mating between *C. eurytheme* female and *C. interior* male was obtained during the same period.

It was impossible to use *C. philodice* for mating, because almost all of my *C. philodice* emerged before any *C. interior* were found in the field.

About 180 pupae of *C. eurytheme* and *C. philodice*, of which larvae were reared on white clover, alfalfa, and vetch at Notre Dame, were brought to the Biological Station on 11 July 1955. The pupae obtained this year were reduced by virus disease to less than half the number expected. Yet 69 females of *C. eurytheme* and *C. philodice*, including "alba" forms of both species, emerged between 12 and 19 July.

Again, matings were tried at Mud Lake and at Elliot's Creek. Fifteen males of *C. interior* were used for matings. The majority of the 69 females which emerged were used for the matings. About 20 females of *C. philodice* were used this year, and attempts by *C. interior* males to mate with *C. philodice* females were clearly observed. However, no interspecific mating was obtained either between a *C. interior* male and a *C. eurytheme* or a *C. philodice* female. Two *C. philodice* males caught in the field mated with *C. philodice* females in the type B cage almost immediately after being introduced into the cage.

The orange female of *C. eurytheme* (W-15-62), which was mated in 1954 to *C. interior*, laid eggs on the leaves of white clover at the Notre Dame greenhouse. Thirty-three eggs were laid on 12 July (group A), 25 on 13 July (group B), 26 from 14–20 July (group C). About 8 eggs were excluded from the above count because they failed to turn red and were presumably infertile. One of the causes of failure to turn red has been observed to be sucking by small insects or mites. It is difficult to determine the egg fertility, but if it is presumed that all eggs which did not turn red were infertile, the percentage of infertile eggs was about 10%.

Groups A and B were reared in the greenhouse, and group C was reared in a rearing room which was kept at 75° F, 75% R.H., and 10 hours fluorescent light illumination a day. The eggs from intraspecific matings of *C. eurytheme* were reared at the same time in both places as controls.

All fertile eggs hatched in groups A and B, and only a few did not hatch in group C. No deaths of larvae were observed in the first and second instar in any of the three groups.

From group A, 26 hybrid males emerged in 6 days (8–13 August) (fig. 1). The developmental rates of these males equalled the developmental rates of 8 control *C. eurytheme* males which emerged from 10 to 18 August. But the developmental rates of the other 7 larvae of group A was extremely slow. Although one of them continued to grow and emerged as a female on 26 August (control—11 *C. eurytheme* females emerged from 9 to 17 August), the others stopped growing at the 3rd or 4th instar.

Sixteen pupae were obtained from group B with ordinary developmental rates. Ten males emerged from these, while six were kept in the refrigerator at about 35° F. These six pupae died in the refrigerator, presumably failing to come out from their pupal case because of low temperature.

Including groups A and B, 15 larvae of the 3rd and 4th instars were counted on 24 August. No larval death had been observed by this time in groups A and B.

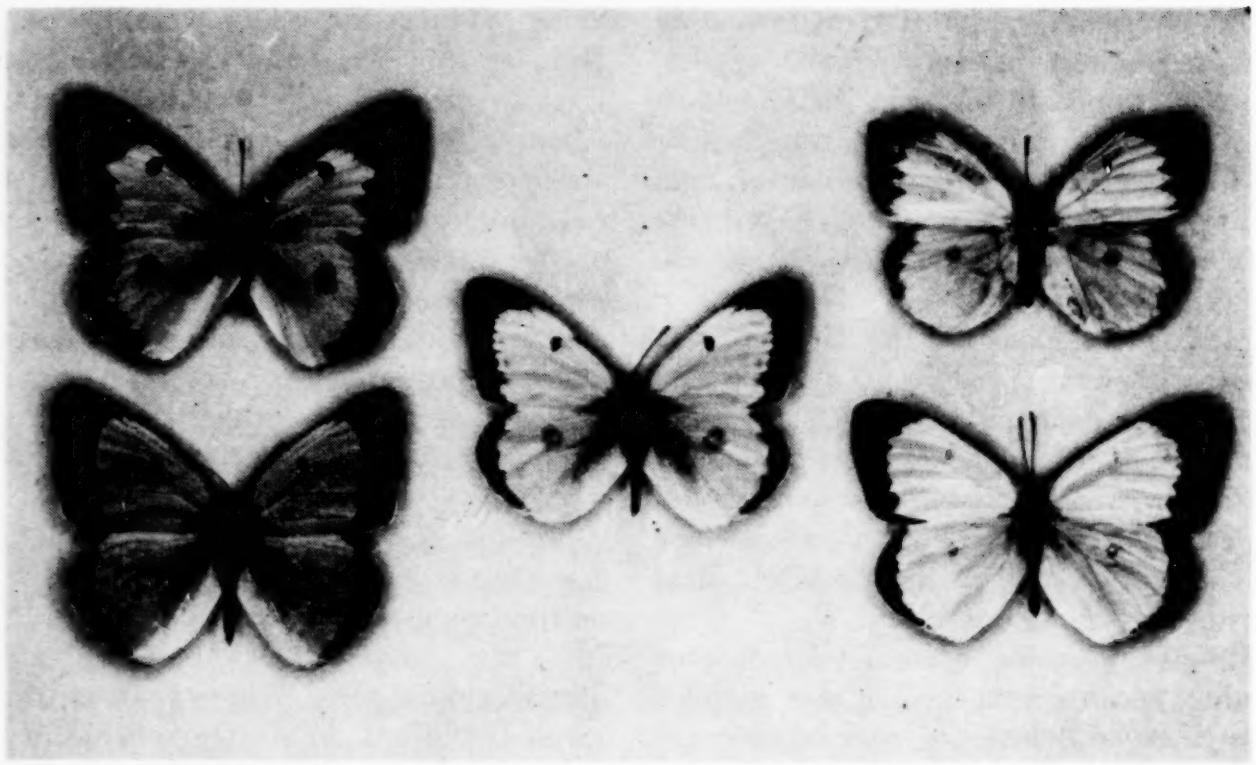

FIG. 1. *Colias eurytheme* (left), *C. interior* (right), and a greenhouse-reared male F_1 hybrid between them (center). Above—females, below—males. Photographed with a yellow-green filter.

In an attempt to break diapause, nine of these larvae were refrigerated at about 35° F from 24 to 31 August and moved to the greenhouse again. They did not feed and died one by one, as did the other six which were not refrigerated.

The larvae of group C, in the rearing room, grew at almost the same rate as the *eurytheme* controls until the 3rd instar, but they stopped feeding at the 3rd instar (a few in 4th instar) and remained largely motionless for about 10 days at the beginning of August. They were moved to the greenhouse on 17 August. They were kept in the greenhouse during the daytime, when the temperature was 80 to 100° F, and were moved to the rearing room at night to prevent chilling. After this was repeated for several days, they began to feed again one by one from 21 to 23 August. After the evening of 24 August, they were kept in the rearing room continuously. Because of this non-feeding stage, the first larva of group C entered the 5th instar 22 days later than the first of the *C. eurytheme* controls. By 24 August the number of larvae had decreased to 12 from the initial number of 26 eggs. The first adult emerged on 5 September and the last one on 2 October. The total number was 10, and all were males. The other two seemed to be growing very slowly, but they died around the middle of October without reaching the 5th instar. From the control brood, reared in the rearing room, 13 males emerged from 11 to 19 August, and 8 females emerged from 14 to 19 August.

Hybrid males were very active in the type B cages and mated readily with orange *C. eurytheme* females. Six backcrosses were obtained within 4 days (9–12 August). About 12 females of orange *C. eurytheme* and about 20 hybrid males were used in the backcross attempts. Three (W-17-22-3, W-17-22-7, W-17-23-4) of the above females laid eggs, while the other three females died without laying eggs about 10 days after their copulations. The egg fertilities are shown in table 1.

The progeny of W-17-22-3 was divided into 2 groups, designated D and E. Group D was reared in the greenhouse, and Group E was reared in the rearing room from the first instar, under the

TABLE 1. *Egg Fertility of Hybrids Between* C. eurytheme *and* C. interior

Female parent	Male parent	Kind of mating	Egg fertility	No. of eggs laid
W-15-62	wild	E × I	90%	93
W-17-22-3	W-15-62-1	E × F	93%	138
W-17-22-7	W-15-62-12	E × F	98%	88
W-17-23-4	W-15-62-15	E × F	0%	50+
W-17-22-7-10 (9)	W-15-62-46	B × F	90%	61
W-17-22-3-4 (7)	W-17-22-3-1 (8)	B × B	99%	154
W-17-22-3-5 (3)	wild	B × E	96%	137
W-17-22-3-12 (7)	W-17-22-3-6 (8)	B × B	98%	170
W-21-54	W-17-22-3-3 (7)	E × B	97%	127
W-17-22-3-9 (3)	W-21-12	B × E	100%	42
W-17-22-3-46 (5)	W-17-22-7-7 (7)	B × B	99%	228
W-17-22-7-28 (8)	W-17-22-7-21 (8)	B × B	96%	100

Explanation of designations: E—*C. eurytheme;* I—*C. interior;* F—F_1 hybrid *C. eurytheme* × *C. interior;* B—backcrossed individual of the above F_1 to *C. eurytheme;* ( )—orange color grades (9—reddest; 0—yellowest).

same conditions as the F_1 larvae. White clover was furnished both groups as a food plant. The progeny of W-17-22-7 was reared in the greenhouse on alfalfa. The developmental rate of the majority of the backcross larvae was the same as that of *C. eurytheme* larvae which were reared as controls, but a few were very slow like the F_1 hybrids, although no larva stopped feeding. In this case the females emerged as early as the males, but the butterflies which emerged last were all females.

Females of Brood W-17-22-3 mated readily with their two brothers, a male of Brood W-17-22-7, and three *C. eurytheme* males. Females of Brood W-17-22-7 also readily mated with two brothers and two males of Brood W-15-62 (F_1 males: W-15-62-34 in group B which was kept in the refrigerator at about 35° F in the pupal stage for about one month; W-15-62-46 in group C). One male of Brood W-17-22-3 mated with a *C. eurytheme* female. The egg fertilities of the above females which laid eggs are shown in table 1. A large number of progeny was produced from the above females. Matings among them were successful.

One yellow *C. interior* female (I-16-1) emerged on the afternoon of 12 September 1955 in the rearing room at Notre Dame. This female was the offspring of a female from Maine. It was immediately placed in a type B cage with 16 wild *C. eurytheme* and 8 wild *C. philodice* males under the fall sun in the greenhouse. Males were very active in the cage, and one *C. eurytheme* male extended its abdomen toward the *C. interior* female. However, no copulation occurred on this day or the two additional days' trial. This female laid a few infertile eggs before it died.

B. *C. Eurytheme* and *C. Philodice* × *C. Alexandra*

Four *C. alexandra* males emerged between 4 and 8 September 1955 in the rearing room at Notre Dame. These were the progeny from a yellow female (A-2) from Gothic. These males were placed in the type B cage with several females of *C. eurytheme* and *C. philodice*. Although *C. alexandra* males were fairly active in the cage, no mating attempt by them was observed, and consequently no mating was obtained.

About 160 pupae of *C. eurytheme* and *C. philodice* were reared in the rearing room of Notre Dame, in the early summer, 1956. Vetch (*Vicia villosa*) was used as the food plant. Although an attempt was made to rear more than 500

pupae, virus disease killed the majority of the larvae before pupation. These pupae were brought to the Rocky Mountain Biological Laboratory on 1 July. Fifty-nine viable females emerged between 3 and 12 July.

C. alexandra and *C. scudderi* were commonly found in the Laboratory region (Gothic). *C. meadii* is usually distributed at higher elevations than Gothic valley, although they appear down there quite often. *C. philodice eriphyle* appears also commonly. From 2 to 15 July, 32 *C. alexandra* males were collected and used for the hybrid matings with Notre Dame females of *C. eurytheme* and *C. philodice* at the outside of the laboratory. The weather was usually fair between 2 and 15 July. *C. alexandra* males were very active in the cages. More than 5 males were usually placed in the cage with some males of *C. scudderi* and *C. meadii*. The supply of newly emerged females was small. Therefore, only a few newly emerged, along with some older females, could usually be used at a time. Neither *C. eurytheme* nor *C. philodice* females mated readily with *C. alexandra* males, although males tried eagerly to mate, extending their abdomens toward females quite often.

On 4 July about 10 *C. alexandra* males and 3 *C. scudderi* males and 2 orange *C. eurytheme* females and 1 yellow *C. philodice* female were placed in a type B cage under the sun. In the afternoon, the yellow *C. philodice* female (P-33-1) which had emerged within 30 minutes, copulated with a *C. alexandra* male, but only for a few minutes.

In the morning of 6 July, one *C. alexandra* male copulated with an orange *C. eurytheme* female which had just emerged, but again for a few minutes. The following observation was made by Dr. C. L. Remington. At 2:00 P.M. 9 *C. alexandra,* 6 *C. scudderi,* and 2 *C. meadii* males were placed with 7 *C. eurytheme* females in a type A cage under the sun. At 2:10 P.M., one "alba" *C. eurytheme* female (P-26-10) which had emerged late in the morning copulated with a *C. alexandra* male. They were isolated immediately from the other butterflies and brought into a cool laboratory room, where they stayed together until 3:50 P.M.

In the morning of 7 July, 6 *C. alexandra* and 6 *C. scudderi* males were placed with 9 *C. eurytheme* and 1 *C. philodice* females under the sun in a type A cage. One orange female copulated with a *C. alexandra* male for a few minutes in this cage. Seven *C. alexandra* males were placed under the sun in a type B cage. Later 2 orange *C. eurytheme* females, just emerged, were put into this cage. One of these females (P-24-8) copulated almost immediately with a *C. alexandra* male. They copulated from 9:25 to 12:00 A.M. In the afternoon, one newly emerged orange female *C. eurytheme* (P-30-8) copulated with a *C. alexandra* male from 2:15 to 5:00 P.M.

In the afternoon of 8 July, 15 *C. alexandra,* 10 *C. scudderi,* and 2 *C. meadii* males were placed under the sun, approximately equally divided into type A and B cages. Ten *C. eurytheme* females of Brood P-26 (6 "alba," 4 orange), from pupae kept in a cool place during the morning, emerged one by one in the afternoon and were placed into type A and B cages immediately. In the B cage 3 copulations between those females and *C. alexandra* males were obtained as follows: "alba" P-26-58 (2:45–5:30 P.M.), orange P-26-64 (3:10–10:50 P.M.), and "alba" P-26-59 (3:20–6:00 P.M.). No copulation was obtained in type A cage.

Among the 6 *C. eurytheme* females which mated with *C. alexandra* males (more than 1 hour), 4 laid eggs while confined on potted white clover. The eggs which were laid by two females (P-26-10 and P-26-59) turned red, but the more than 20 eggs laid each by P-26-64 and P-30-8 did not turn red and therefore were presumed infertile. The other 2 females did not lay any eggs: P-24-8 died the day after copulation (one

TABLE 2. *Egg fertility of hybrid* C. eurytheme × C. alexandra, C. philodice × C. alexandra, *and* C. eurytheme × C. philodice

Female parent	Male parent	Kind of mating	Egg fertility	No. of eggs laid
P–26–10	wild	E × A	99%	598
P–26–59	wild	E × A	100%	7
P–26–64	wild	E × A	0%	20+
P–30–8	wild	E × A	0%	20+
E–8–37	wild	E × A	96%	381
E–8–38	wild	E × A	30%	47
E–16–18	wild	E × A	98%	126
E–4–39	wild	E × A	99%	199
E–7–50	wild	P × A	100%	2
W–12–76–11	wild	E × P	97%	67
P–62–9–7–10	P–76–8	E × P	52%	315
P–62–9–7–10–2	P–62–9–7–10–11	F × F	95%	206
P–62–9–7–10–9	P–62–9–7–10–6	F × F	68%	25
P–62–9–7–10–17	P–62–9–7–10–5	F × F	100%	8
P–62–9–7–3–4	P–62–9–7–10–12	E × F	84%	81
P–62–9–7–3–6	P–62–9–7–10–12	E × F	67%	3

Explanation of designations: E—*C. eurytheme;* A—*C. alexandra;* P—*C. philodice;* F—F_1 hybrid *C. eurytheme* × *C. philodice.*

spermatophore and some immature eggs were found in its abdomen), and P-26-58 lived for more than 2 weeks after copulation without laying any eggs. This female was dissected later and a spermatophore and many presumably mature eggs were found in its abdomen, but no red egg was found. The total number of eggs laid by P-26-10 between 8 and 20 July was 598, and 6 of them did not turn red. P-26-59 laid only 7 eggs on 10 July, all of which turned red (table 2).

The white clover leaves, on which 130 fertile eggs of P-26-10 and 7 fertile eggs of P-26-59 were laid, were detached from the plants and kept in plastic containers. Most of these eggs hatched and the first instar larvae were placed on white clover leaves which had their stems in water. However, most of them failed to survive beyond the first instar. Only 6 of Brood P-26-10 and none of Brood P-26-59 reached the second instar. The other eggs of Brood P-26-10 were kept on white clover leaves on which they were laid, without detaching the leaves from the plants. In this case, the deaths during the egg and the first instar were both few. Unfortunately, virus diseases appeared during their larval stage, and almost all of the larvae were lost before pupation. One larva from the eggs laid on 8–9 July pupated on 3 August and emerged on 10 August as a male (fig. 2). A second male emerged on 27 August. About 10 more larvae reached the 5th instar before 27 August, but all of them died during the 5th instar or pupal stage. Both males which emerged were healthy; however, to mate them was impossible; because of the severe virus epidemic, no virgin female of any *Colias* species was available at that time.

The above rearing was carried out in the laboratory room at Gothic. The room temperature during the daytime was around 70–80° F. To prevent the temperature drop at night, one electric heater was used from 15 July. The morning sun shone in the room until a little before noon. From 25 July the room was lighted for 14 hours a day by fluorescent and incandescent lamps.

On 27 August, 27 3rd instar larvae were counted; none of the larvae had fed during the previous few days. They survived the air trip to New Haven. At the window side of Gibbs Laboratory, they

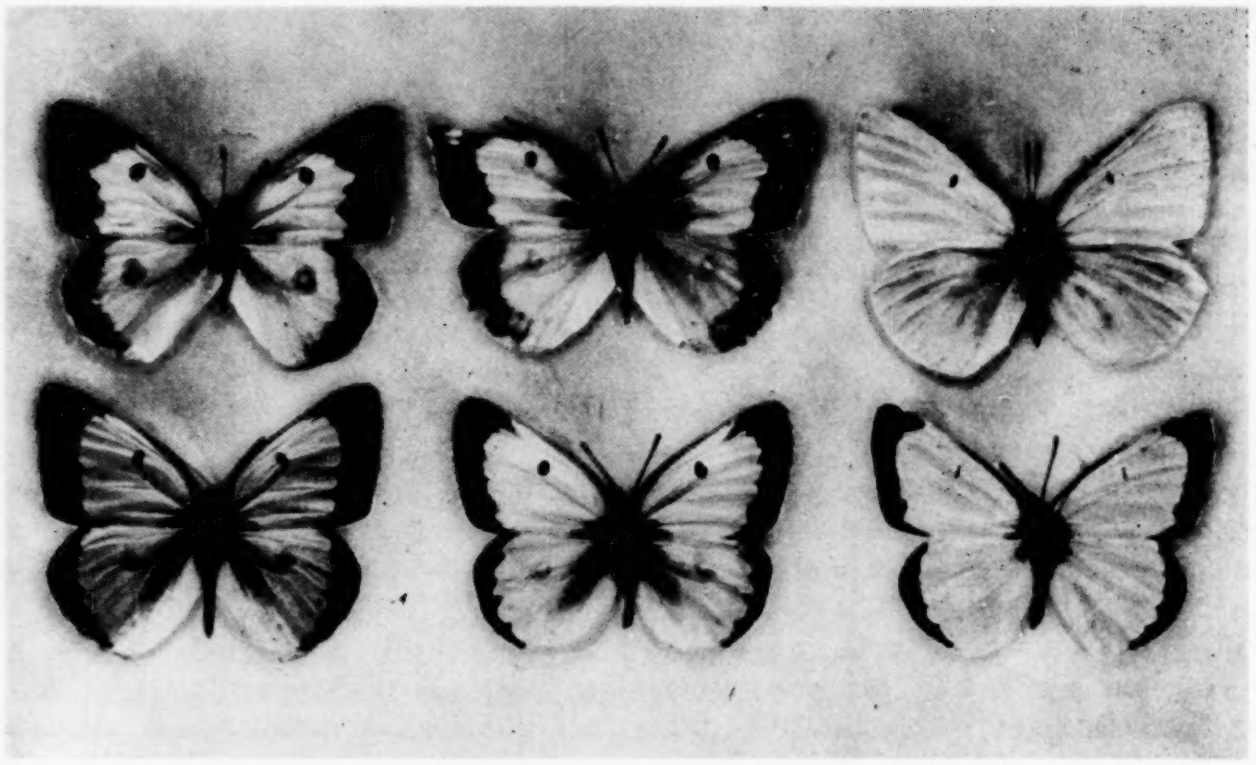

FIG. 2. *Colias eurytheme* (left), *C. alexandra* (right), and F_1 hybrids between them (center). Above—females, below—males. Photographed with a yellow-green filter.

started rapidly, one by one, to eat white clover or vetch again, after a day or so. Several days later, some were moved to the rearing room. Three reached the 5th instar and another 3 reached the 4th instar during the month of September. However, virus disease killed them all. In the beginning of October, only one larva survived, and it was resting on the vetch leaves without feeding. On 5 October when bright fluorescent lights were set in the rearing room, this larva was moved to a new vetch seedling and placed under the new light. On 12 October it suddenly started to eat vetch leaves very actively and molted on 16 October (4th instar). It pupated on 22 October and emerged on the morning of 29 October as a female (fig. 2).

This female was immediately placed under the bright sun at the window side of the laboratory in a type A cage in which 4 laboratory-reared and 5 wild males of *C. eurytheme* had been placed early in the morning. Four laboratory-reared and 2 wild males of *C. eurytheme* were added later. At least two different males extended their abdomens toward the female attempting to mate. This behavior was observed at least 10 times during the day. However, the female did not respond at all, and no mating had occurred by sunset. (One *C. eurytheme* control female was placed in this cage in the afternoon; it mated immediately with one of its sibs.) On the next day, and for several more times with a few days interval the hybrid female was placed with *C. eurytheme* and *C. philodice* males with no successful mating. *C. philodice* males also extended their abdomens to the female, attempting to mate.

About 650 pupae of *C. eurytheme, C. philodice,* and hybrids between these two species were obtained in the rearing room of the Gibbs Laboratory in the early summer of 1957. Vetch was used as the food plant. These pupae were brought to the Rocky Mountain Biological Laboratory on 28 June by air. Deaths of the pupae before emergence were very few. However, because of an extraordinary delay of about one month in the field season and in appearance of *C. alexandra, C. scudderi,* and *C. meadii* at Gothic valley, most of the pupae emerged before

the appearance of the above species. About 100 pupae of *C. eurytheme* and *C. philodice* were kept in a refrigerator. They were brought out after *C. alexandra* appeared in the field. However, due presumably to the refrigeration, a large number of these pupae failed to emerge or to extend their wings after their emergences.

On 22 July, 1 *C. alexandra* male was caught at Gothic. It was immediately (at 11:40 A.M.) placed in a type A cage under the bright sun with 10 *C. eurytheme* females which had emerged between 19 and 22 July. One "alba" female (E-8-37), which had emerged in the morning, mated with this *C. alexandra* male and remained *in copulo* from 11:50 A.M. to 2:10 P.M. Another *C. alexandra* male was caught in the afternoon at Gothic. It was immediately placed, with 3 *C. eurytheme* females which had emerged within one hour, in a type B cage under the bright sun. One "alba" female (E-8-38) mated with this male from 1:10 to 7:30 P.M.

In the morning of 24 July, one of two *C. alexandra* males mated on 22 July was placed in a type A cage with about 30 *C. eurytheme* females. Another male was placed with about 20 *C. philodice* females in another type A cage. These females were all more than 2 days old. Both cages were exposed in sunlight for about 2 hours. In both cages, males extended their abdomens toward females very often. But no copulation occurred. Later 3 new *C. eurytheme* emerged. They and 5 *C. philodice* females and 2 *C. eurytheme* females (both species more than one day old) were placed with the above 2 *C. alexandra* males in a type A cage under the sun. A *C. alexandra* male mated previously with E-8-37 mated again with a newly emerged *C. eurytheme* orange female (E-16-18) from 11:50 A.M. to 2:30 P.M.

Another successful mating (more than 1 hour) was obtained on 28 July between a just-emerged *C. eurytheme* orange female (E-4-39) and a just-collected *C. alexandra* male. The last two matings were obtained from females from refrigerated pupae. No more mating was tried between newly emerged *C. eurytheme* females and *C. alexandra* males. A total of 6 *C. alexandra* males were used in all, before the above 4 matings were obtained.

On 30 July, 3 *C. philodice* females, which had emerged early in the morning, were placed with 5 *C. alexandra* males (the same males used previously) in a type B cage at 8:15 A.M. under the sun. One "alba" female (E-7-45) mated with a *C. alexandra* male from 9:00 A.M. to 2:30 P.M. This male had previously mated with a *C. eurytheme* female (E-4-39).

On 3 August, 2 newly emerged *C. philodice* females were placed with 7 *C. alexandra* males under the sun in a type B cage at 8:00 A.M. One male, collected on 1 August, mated with a yellow female (E-7-50) from 9:37 A.M. to 11:53 A.M.

A few more attempts to obtain matings between *C. alexandra* males and a large number of *C. eurytheme* and *C. philodice* females more than one day old gave no mating. However, one of these males mated in a cage with a wild *C. alexandra* female, which was virgin (its eggs did not turn red). This female laid fertile eggs after copulation.

All 4 *C. eurytheme* females which mated with *C. alexandra* laid fertile eggs (turned red) while confined on potted white clover. Table 2 shows the egg fertility. No death of fertile eggs before hatch was observed in the above 4 broods. Most of the eggs hatched about 4 days after laying. However, some of the eggs had 5 or 6 days of egg period.

The larvae were reared in the laboratory room at Gothic. The room temperature was kept between 75 and 85° F with an electric heater. Fluorescent and incandescent lamps were lighted constantly except in the morning, when sun shone in the room. Most of the larvae were killed by virus diseases. The first and second male emerged on 18 August from Broods E-8-37 and E-16-18. One

male from Brood E-16-18 emerged on 20 August and two males from Broods E-8-37 and E-16-18 emerged on 21 August. About 15 more larvae from all 4 broods reached the 5th instar or pupal stage. However, virus diseases killed them all. The development of the above larvae was continuous, and the rates were approximately the same with *C. eurytheme* reared at the same time. However, the developmental rates of many other hybrid larvae were very slow and some of them stopped feeding and became the hibernating form at the 3rd instar. On 3 September, 2 hibernating larvae of Brood E-8-37 and 9 hibernating larvae of Brood E-16-18 were counted. They were kept in a cool place, but died before winter because of disease or unsuitable conditions. These were probably females.

A *C. philodice* yellow female (E-7-30), mated with *C. alexandra,* was confined on potted white clover. It laid 2 fertile eggs (turned red) 3 days after copulation (table 2). This female died next day and laid no more eggs. It was dissected later, and 1 spermatophore and a few presumably mature eggs were found in its abdomen. The two fertile eggs remained red and dried up without changing their color. A *C. philodice* "alba" female (E-7-45), mated with *C. alexandra,* lived for a few days but laid no egg, although it was confined on potted white clover. It was dissected later, and 1 spermatophore and a few presumably mature eggs were found in its abdomen.

C. *C. Eurytheme* and *C. Philodice* × *C. Meadii*

Thirteen *C. meadii* males were collected in the vicinity of Gothic and were used in attempts to get hybrid matings with Notre Dame females of *C. eurytheme* (and a few females of *C. philodice*) between 6 and 15 July in 1956 at the Rocky Mountain Biological Laboratory. *C. meadii* males could not be obtained in large numbers while these fresh females were emerging. Two males were used on 6 July without success (see section B). The same males were used again on 8 July. These males were very active, like *C. alexandra* males in the cage (see section B). However, only *C. alexandra* males mated with the *C. eurytheme* females, although males of both species were in the same cage. At 3:30 P.M. all *C. alexandra* males were removed from the type B cage, and 2 *C. meadii* and 10 *C. scudderi* males were placed in the cage with a few *C. eurytheme* females which had emerged the same afternoon. At 3:55 P.M. one "alba" female (P-26-60) copulated with a *C. meadii* male, and they stayed together until 6:00 P.M.

After 8 July only a few females emerged. Although matings were tried for a few more days, using all 13 *C. meadii* males, most of which were collected on 8 and 9 July, no more copulation was observed.

Female P-26-60, which had mated with a *C. meadii* male, lived for more than two weeks, but it did not lay any eggs. It was confined on white clover. This female was dissected, and a spermatophore and many presumably mature white eggs were found. However, no red egg was found.

One male *C. meadii,* from eggs laid by a Gothic *C. meadii* female, emerged on 1 October 1956 in the rearing room at the Gibbs Laboratory. It lived for a few weeks and was active in the cage, but it did not mate with any of several *C. philodice,* one *C. eurytheme,* or three females of its own sibs.

Three females of *C. meadii,* which were the sibs of the above male, emerged in the rearing room at the end of September. Although the weather and the time of emergence were not suitable for using these females for matings at the moment of their emergences, many wild and laboratory-reared males of *C. eurytheme* and *C. philodice* and one male of *C. meadii* were placed with them for several days. No copulation was obtained among them.

Because of the delayed appearance of *C. meadii* in the vicinity of Gothic valley, no mating attempts were possible in 1957.

D. *C. Eurytheme* and *C. Philodice* × *C. Scudderi*

Twelve *C. scudderi* males, collected at Gothic, were used in attempts to get matings with Notre Dame females of *C. eurytheme* and *C. philodice* between 4 and 15 July in 1956 at the Rocky Mountain Biological Laboratory. Usually more than 5 *C. scudderi* males were placed in each cage, when matings were being tried between *C. eurytheme* and *C. philodice* females and *C. alexandra* and *C. meadii* males, as has been described in the previous sections. These *C. scudderi* males were not as active as *C. alexandra* nor *C. meadii* males. Yet they occasionally tried to mate with *C. eurytheme* and *C. philodice* females, extending their abdomena toward these females. However, no actual copulation took place during the whole period.

One *C. scudderi* male, from an egg which was laid by a Gothic *C. scudderi,* emerged on 28 September 1956 in the rearing room at the Gibbs Laboratory and lived for a few weeks. Several females of *C. philodice* and a *C. eurytheme* female were used for attempts at hybrid matings at the window side of the laboratory, without success.

On 4 August 1957, 5 wild *C. scudderi* males, collected between 30 July and 2 August, were placed under the sun in a type B cage with 4 *C. eurytheme* orange females from 10:30 A.M. to 12:30 P.M. Three of these females had emerged the same morning and the other on 3 August. All of them came from refrigerated pupae. These *C. scudderi* males were fairly active in a cage and tried to mate, often extending their abdomens toward females. One male copulated with a female. They did not try to disengage immediately after copulation, but they separated after about 5 minutes. This female died next day without laying any egg. One of the above males mated in a cage with a wild *C. scudderi* female, which was virgin (its eggs collected before the cage mating did not turn red). This female laid fertile eggs after copulation.

E. *C. Eurytheme* × *C. Philodice*

Since intraspecific matings among caged *C. eurytheme* and *C. philodice* are not always successful, it is difficult to assess their interspecific matability. In general, it appears that interspecific matability between *C. eurytheme* and *C. philodice* is lower than their intraspecific matabilities in the writer's many experiments, although these experiments were usually not expressly designed to test this.

One experiment was carefully carried out to test inter- and intraspecific matabilities. On 7 November 1956, at the Gibbs Laboratory, 5 pairs of *C. eurytheme* and 5 pairs of *C. philodice* were placed near the windows of the laboratory, in bright sunshine, in one type A cage. These butterflies were all laboratory-reared individuals, and both males and females were more than 24 hours old. From 9:00 A.M. to 2:30 P.M., 1 intraspecific mating of *C. eurytheme* and 2 intraspecific matings of *C. philodice* were obtained. Copulating pairs were isolated. At 2:30 P.M., 1 pair of *C. eurytheme* and 2 pairs of *C. philodice* were added. All were laboratory-reared individuals. The *C. eurytheme* female was several hours old, but the other 5 new butterflies were more than one day old. One more intraspecific mating of *C. philodice* was obtained. On the next day, 4 pairs of *C. eurytheme* and 4 pairs of *C. philodice* were placed in the same cage at the windows of the laboratory. The weather was fair. Again all were laboratory-reared individuals more than one day old. From 8:50 A.M. to 12:30 P.M., 2 intraspecific matings of *C. eurytheme* and 2 intraspecific matings of *C. philodice* were obtained.

In summary, using 7 pairs each of *C. eurytheme* and *C. philodice,* 3 intraspecific matings of *C. eurytheme* and 5 intraspecific matings of *C. philodice* were ob-

tained, but no interspecific matings were obtained. In this experiment, the difference between inter- and intraspecific matabilities is clear, since intraspecific matabilities of both species were high. The probability of successive occurrence of 8 intraspecific matings, under the condition in which both intra- and interspecific matings should occur at random, is less than 1% ($(\frac{1}{2})^8 = 0.004$).

The egg fertility which the writer obtained from a *C. eurytheme* female (W-12-76-11) × wild *C. philodice* male at Notre Dame in 1954 was 97% (table 2). The developmental rates of the larvae from these hybrid eggs were approximately the same as for *C. eurytheme* and *C. philodice* at the same conditions (75° F and 10 hrs. light a day).

One mating was obtained on 21 December 1956, under the fluorescent light in the rearing room at the Gibbs Laboratory, between a *C. eurytheme* female (P-62-9-7-10, a F_3 individual from continuous sib-matings from local orange *C. eurytheme* female P-62) and a *C. philodice* male (P-76-8, a F_1 individual from local yellow *C. philodice* female, P-76). 315 eggs were obtained from this mating, with 51.7% egg fertility (table 2). The larvae from these eggs were kept in the rearing room. The reduced egg hatchability, virus diseases, and other factors greatly reduced the number of adults obtained from these eggs. The developmental rates of the larvae were approximately the same as for *C. eurytheme* and *C. philodice* reared at the same conditions. A total of 10 females and 13 males emerged. They mated readily with each other under the fluorescent light in a type B cage, and 4 matings were obtained. One male also mated with two *C. eurytheme* females.

Three F_1 hybrid females which had mated with their sibs laid fertile eggs, while the fourth did not lay any eggs. This fourth female was dissected when it died, two weeks after its copulation. No visibly developed egg was present, although one spermatophore was found. The egg fertilities of the above three fertile females are shown in table 2. The reduction of egg hatchability was not as marked as with the F_1 eggs. However, the combination of virus disease and probable hereditary lethal defects again prevented the obtaining of many adults from the F_2 hybrid larvae, which were reared under the same conditions as the F_1. In all, 12 females and 9 males emerged.

The egg fertilities of the 2 *C. eurytheme* females mated with the F_1 male are shown in table 2. The larger of these backcross broods was reared in the rearing room. The reduction of egg hatchability was not severe, and the larvae grew fairly well. Ten females and 16 males were obtained.

2. Genetics of Interspecific Characters

A. Hybrids between *C. Eurytheme* and *C. Interior*

There was only one conspicuous difference observed among shape or coloration of egg, larva, and pupa of the F_1 hybrids in comparison with the control (*C. eurytheme*). Namely, in the 4th instar, the hybrids showed a distinct, but not intense, crimson line along the spiracular fold, while *C. eurytheme* had a white line. In the 5th instar, the hybrids showed a very clear crimson line, but *C. eurytheme* showed an orange line. This seems to indicate blending-type inheritance of this larval coloration.

F_1 hybrid larvae did not go into hibernation in the summer greenhouse conditions in which *C. interior* larvae began hibernation. However, hybrid larvae entered this non-feeding stage under conditions of 75° F and 10 hours light a day, in which *C. eurytheme* larvae grow continuously (see section 1). Therefore, F_1 hybrid larvae seem to show characteristics somewhat intermediate between *C. eurytheme* and *C. interior* concerning hibernation, which definitely involves hereditary factors.

The characteristics of F_1 adult male hybrids reared in the greenhouse are as follows (see fig. 1):

a) The size is the same as for *C. eurytheme* which emerged in the greenhouse during the same period.

b) The pterine coloration is intermediate between *C. eurytheme* and *C. interior*. The orange pigment is distributed over most of the upper side of both wings. The individual variation of intensity of orange color is very slight among the 26 males. The orange color grade is 5, and rather closer to 4 than to 6.

c) Submarginal dark spots (lacking in *C. interior* but present in *C. eurytheme*), and the dark spot on the costa of the hind wing (inconspicuous in *C. interior* but prominent in *C. eurytheme*) exist in the hybrid.

d) The hind wing discal spot is double (usually single in *C. interior* and usually double in *C. eurytheme*).

e) The pink fringes are wider than those of *C. eurytheme* and closer to the width and pinkness of *C. interior*.

Unfortunately the wings of the one hybrid female failed to expand. Although it is very difficult to determine the orange color grade on crumpled wings, it seems to show intermediate coloration (grade 4 or 5). No reduction of the black borders of the wings was observed. The greater reduction of black borders is characteristic of *C. interior* female.

All of the larvae of the F_1 hybrids which showed the same developmental rates as *C. eurytheme* emerged as males. These made up more than half of the F_1 brood. Only one of the larvae with the slower developmental rate emerged and this was a female. Hence it may be that all or most of the slow-growing larvae were females and these females may be showing the character of the slow-growing *C. interior*.

The characteristics of F_1 males reared in the rearing room (75° F, 10 hours light a day) are as follows:

a) The size is smaller than that of the males which emerged in the greenhouse, but it is equal to the *C. eurytheme* controls.

b) The orange color grades are mainly 3 (8 of grade 3, 1 of grade 4, and 1 of grade 5) and the reduction of the orange color towards the tip of the upper fore wing is usually prominent. Orange color grades of the control *C. eurytheme* are the following: 7 of grade 9, 2 of grade 8, and 2 of grade 7. The reduction of orange color toward the tip of the fore wing of the upper side among them is also prominent.

c) The fuscous dusting on the under side of the hind wing is somewhat prominent. This dusting is not found at all or found very slightly among *C. interior* males which the writer collected in Cheboygan. However, it is found among *C. interior* of more northern regions (Maine, Canada).

The characteristics of the progeny of F_1 hybrids backcrossed to *C. eurytheme* are as follows:

a) The orange color grades are shown in table 3. They are spread between the color grades of *C. eurytheme* and F_1 hybrids with some lower grades than the F_1. These lower color grades may be the result of the shortening of the day length towards the fall season.

b) The black borders in females are as wide as typical *C. eurytheme* in all greenhouse-reared females. Some reduction was observed among the rearing room females. However, this reduction occurred to the same extent also in a *C. eurytheme* brood in the rearing room under the same conditions and is therefore presumed to be environmental.

The sib matings among the above backcross progeny which were reared in the rearing room also gave the wide range of orange color grades. However, no pure yellow individuals were produced, although one female had grade 1 (table 5). The reduction of black borders in females was also present to the same extent as in rearing room reared backcross females.

B. Hybrids Between *C. Eurytheme* and *C. Alexandra*

The characteristics of 2 F_1 hybrid males between *C. eurytheme* and *C. alexandra* obtained in 1956 are as follows (see fig. 2):

a) The orange pigment is distributed over most of the upper side of both wings. The orange color grade is 4.

b) The discal spot on the hind wing is orange on the upper side. This spot is close to red in *C. eurytheme,* although many specimens have orange spots as in the above F_1 hybrids. *C. alexandra* usually has yellow spots, but sometimes has slightly orange spots.

c) These spots of both F_1 males have the ring of brownish pink and fuscous scales with silver center on the underside, just as in *C. eurytheme. C. alexandra* has only silver scales and usually lacks this ring. Some *C. alexandra* specimens have this ring like *C. eurytheme,* but it is not as prominent.

TABLE 3. *Color grades of hybrid* C. eurytheme × C. interior

Parents		S	C	R	Color grades of offspring											
					9	8	7	6	5	4	3	2	1	0	A	Total
Female	W–15–62	E	9	g	0	0	0	0	1	0	0	0	0	0	0	1
Male	wild	I	0		0	0	0	0	26	0	0	0	0	0	0	26
Female	W–15–62	E	9	r	0	0	0	0	0	0	0	0	0	0	0	0
Male	wild	I	0		0	0	0	0	1	1	8	0	0	0	0	10
Female	W–12–76–11	E	9	r	0	0	0	0	0	5	31	3	0	0	0	39
Male	wild	P	0		0	0	0	0	0	1	11	2	0	0	0	14
Female	W–17–22–3	E	9	g	1	3	4	0	1	0	4	1	0	0	0	14
Male	W–15–62–1	F	5		4	5	5	0	0	0	0	0	0	0	0	14
Female	W–17–22–3	E	9	r	0	0	1	0	2	2	1	0	0	0	0	6
Male	W–15–62–1	F	5		0	0	1	1	3	0	0	5	0	0	0	10
Female	W–17–22–7	E	9	g	6	7	6	1	0	0	0	0	0	0	0	20
Male	W–15–62–12	F	5		10	7	6	4	0	0	0	0	0	0	0	27
Female	W–17–22–7–10	B	9	r	1	1	1	0	1	0	0	0	0	0	0	4
Male	W–15–62–46	F	3*		0	0	6	2	3	1	0	0	0	0	0	12
Female	W–17–22–3–5	B	3	r	0	2	11	12	6	2	0	0	0	0	0	33
Male	wild	E	9		0	6	9	5	10	6	2	0	0	0	0	38
Female	W–17–22–3–9	B	9	r	2	0	1	0	0	0	0	0	0	0	10	13
Male	W–21–12	E	9		12	4	0	0	0	0	0	0	0	0	0	16
Female	W–21–54	E	9	r	6	10	16	13	4	0	0	0	0	0	0	49
Male	W–17–22–3–3	B	7		0	2	35	6	0	0	0	0	0	0	0	43
Female	W–17–22–3–4	B	7	r	0	0	2	3	7	3	4	0	0	0	0	19
Male	W–17–22–3–1	B	8		0	1	1	4	4	1	0	0	0	0	0	11
Female	W–21–55	E	9	r	8	30	11	1	0	0	0	0	0	0	0	50
Male	W–17–22–3–7	B	8		2	67	27	2	0	0	0	0	0	0	0	98
Female	W–17–22–3–46	B	5*	r	0	0	3	6	4	2	0	2	1	0	0	18
Male	W–17–22–7–7	B	7		0	2	9	1	3	2	5	3	0	0	0	25
Female	W–17–22–7–28	B	8	r	0	1	0	0	1	0	0	0	0	0	0	2
Male	W–17–22–7–21	B	8		0	0	1	1	2	0	1	0	0	0	0	5
Female	W–17–22–7–31	B	8	r	0	0	1	1	0	0	0	0	0	0	0	2
Male	W–17–22–7–24	B	8		0	1	0	0	1	0	0	0	0	0	0	2
Female	W–17–22–3–12	B	7	r	0	1	0	1	3	0	0	0	0	0	0	5
Male	W–17–22–3–6	B	8		0	1	3	2	0	3	1	0	0	0	0	10

Explanation of designations: S—species or kinds of hybrids, E—*C. eurytheme*, I—*C. interior*, P—*C. philodice* (to compare with F_1 hybrid *C. eurytheme* × *C. interior*), F—F_1 hybrid *C. eurytheme* × *C. interior*, B—backcrossed individuals of the above F_1 to *C. eurytheme;* C—color grades of the parents, *—color grades which were obtained in the rearing room kept at 75° F., 10 hours light; R—rearing place, g—greenhouse, r—rearing room kept at 75° F, 10 hours light a day, 75% R.H.; color grades—color grades of Hovanitz (9—most red, 0—yellow), A—"alba" female.

d) This spot is double in one hybrid specimen and single in the other. This spot is usually double in *C. eurytheme,* although specimens which have single spot are not rare. In *C. alexandra* this spot is single almost always, yet double spots are found too.

e) The wing fringes are almost as pink as in *C. eurytheme.* The fringes of *C. alexandra* are pale yellow, although they are pinkish occasionally.

f) The black borders of the wings are wider than in typical *C. alexandra* and not narrower than those of many *C. eurytheme,* although some *C. eurytheme* have wider borders than these hybrids.

g) The dusting of fuscous scales in the underside of the wings is prominent and gives a somewhat grayish-green color, as in *C. alexandra.* This dusting is seen quite often also in *C. eurytheme,* but it gives a more brownish color than the grayish-green of *C. alexandra.*

h) Submarginal dark spots and the dark spot on the costa on the underside of the wings are as prominent as in *C. eurytheme.* These spots are usually lacking in *C. alexandra,* although they are seen occasionally.

The characteristics of 5 F_1 hybrid males from 2 broods (3 of E-16-18 and 2 of E-8-37) in 1957 are as follows in comparison with 1956 males.

a) The orange pigment is distributed over most of the upper side of both wings and the orange color grade is 4 in 3 individuals. The other 2 individuals (E-16-18-1 and E-16-18-2) have some reduction of the orange color towards the tip of the upper fore wings and their orange color grade is 3.

b) The discal spots on the hind wings are single in E-8-37-1 and E-8-37-2 and are double in the other 3 individuals of Brood E-16-18.

c) The dusting of fuscous scales in the underside of the wings is not so prominent as in 2 1956 hybrids, but it also gives a somewhat grayish-green color.

e) Submarginal dark spots and the dark spot on the costa on the underside of the wings are not so prominent as in 2 1956 hybrids, but they are within the range of variation in *C. eurytheme.*

f) The other characteristics are the same as the 2 1956 males.

The characteristics of 1 F_1 hybrid female which is the sib of the 2 1956 F_1 hybrid males are as follows (see fig. 2):

a) The orange pigment is distributed over most of the upper side of both wings. The orange color grade is 4.

b) The hind wing discal spot is orange on the upper side, and the spot is double, although the second one is very small. On the underside, the ring of brownish pink and fuscous scales is clear and has a prominent silver center in the larger spot.

c) The black borders of the wings are wide and indistinguishable from usual *C. eurytheme* females. The borders on the hind wings are rather wider than in usual *C. eurytheme* females. The reduction of the black borders in pure *C. alexandra* female is normally very pronounced.

d) The wing fringes are almost as pink as in *C. eurytheme.*

e) The dusting of fuscous scales on the underside of the wings is prominent and gives a somewhat grayish-green appearance.

f) Submarginal dark spots and the dark spot on the costa are as prominent as in usual *C. eurytheme.* These spots are often not very clear in *C. eurytheme* females, and are usually lacking in *C. alexandra.*

C. Female Presumed F_1 Hybrid Between *C. Philodice* and *C. Alexandra*

One female which the writer collected at Gothic on 19 July 1956 combined characteristics of *C. philodice* and *C. alexandra.* Its black borders of the wings are as wide as in *C. philodice,* and the fuscous dusting of the underside gives a grayish-green color as in *C. alexandra.* The ground coloration is yellow. The hind wing discal spot is yellow on the upper side. Submarginal dark spots and the dark spot on the costa are not so prominent as in *C. philodice.* The wing fringes appear to be yellow, although many of the scales are gone. The fringes of *C. philodice* are pink.

It laid 47 eggs on white clover (fertility—96%). Although larvae emerged from almost all the fertile eggs, many were killed before the 3rd instar by virus diseases. One reached the 5th instar while others stopped feeding in the 3rd instar. However, all of them were killed also by virus diseases without having another molt.

D. Hybrids Between *C. Eurytheme* and *C. Philodice*

The progeny were reared at Notre Dame from 2 wild hybrids between *C. eurytheme* and *C. philodice,* from 1 *C.*

TABLE 4. *Color grades of hybrid* C. eurytheme × C. philodice

Parents		C	Color grades of offspring												
			9	8	7	6	5	4	3	2	1	0	A	T	Total
Female	W–24	4	0	0	0	0	2	5	2	0	0	4	5	9	18
Male	wild	?	0	0	0	0	0	7	0	0	0	12		7	19
Female	W–69	0	0	0	0	0	2	0	0	1	0	2	9	3	14
Male	wild	?	0	0	0	0	5	4	0	0	0	3		9	12
Female	W–69–8	2	0	0	0	0	0	0	4	1	1	8	0	6	14
Male	wild	0	0	0	0	0	8	3	0	0	0	20		11	31
Female	W–69–12	5	0	0	0	0	0	0	4	1	0	2	9	5	16
Male	wild	0	0	0	0	0	8	3	0	0	0	12		11	23
Female	W–77	3	0	0	0	0	0	0	1	6	6	8	0	13	21
Male	wild	?	0	0	0	1	3	1	6	1	0	5		12	17
			Total females									24	23	36	83
			Total males									52		50	102
			Total									76	23	86	185

80°F, 15 hours light a day, except W–24.
C—color grades of parents, A—"alba" female, T—total individuals with some orange coloration.
χ^2 test for 1 : 1 ratio

males—$\chi^2 = 0.0392$	$.90 > P > .80$
females—$\chi^2 = 2.4000$	$.20 > P > .10$
both sexes—$\chi^2 = 0.6172$	$.50 > P > .30$

philodice female whose mate was apparently a hybrid, and from 2 laboratory-reared hybrid females. The wild females were collected at Notre Dame. The results are shown in table 4. The off-spring from the female (W-24) were reared in the greenhouse in the summer of 1954. The others were reared in the rearing room in 1955. These are all backcrosses to yellow genotypes.

One of the yellow females of Brood W-69-8 mated with a wild yellow *C. philodice* male and produced a yellow brood. This brood consisted of 2 yellow and 5 "alba" females and 8 yellow males.

The orange color grades of the F_1 hybrid brood, P-62-9-7-10; the F_2 hybrids broods, P-62-9-7-10-2, P-62-9-7-10-9, and P-62-9-7-10-17; and the brood, P-62-9-7-3-4, which was a backcross of F_1 to *C. eurytheme* female, are shown in table 5. All of them were reared in the rearing room at the Gibbs Laboratory. The width of the black borders in both sexes in the F_1 are more or less intermediate between wide *C. eurytheme* and narrow *C. philodice* and the width in F_2 and backcross seem to be parallel with the orange color grades; that is, the higher the color grades the wider the border.

DISCUSSION

There are some reports of interspecific hybrids in nature between various species of *Colias*. However, the visible characteristics of many species of *Colias* often overlap, and it is also quite possible that mutants cause one species to resemble another in nature, as in yellow hind wing discal spot in *C. philodice* (Remington, 1955) and *C. erate poliographus* (Komai and Ae, 1953; Ae, 1957). *C. philodice* and *C. erate poliographus* usually have an orange discal spot, while many other *Colias* species typically have the spot yellow, as in *C. alexandra*. For these rea-

sons, the writer believes that reports of natural hybrids require comparison with unquestionable laboratory-produced hybrids in order to be acceptable.

As far as the writer knows, among *Colias* only interspecific hybrids between *C. eurytheme* and *C. philodice* have been studied extensively in the field and laboratories by Gerould (1943, 1946), Hovanitz (1943, 1944a, 1944b, 1948, 1949), and Remington (1954) before the writer's work. Whether *C. eurytheme* and *C. philodice* are really two different species is still an open question. This point will be discussed later.

Gerould (1943) found a decided lack of cross-pairing affinity between male *C. eurytheme* and female *C. philodice*. On the other hand, Hovanitz (1944a) concluded that sterility and sexual apathy are the result of change from the normal larval food of the "races" (he treated *C. eurytheme* and *C. philodice* as races of *C. chrysotheme* Esper in the Old World). Hovanitz (1949) also obtained 21 copulating pairs among *C. eurytheme, C. philodice,* and their hybrids in a mixed population in the field. The 21 pairs were classified as 16 intraspecific matings of *C. eurytheme,* 1 interspecific mating between *C. eurytheme* and *C. philodice,* and 4 matings between *C. eurytheme* and hybrids. He concluded that the actual frequency of the mating combinations in the field was very similar to the proportion expected in the basis of relative abundance in the field of the various types and that mating appears to be at random.

In contrast, mating experiments among 6 different species of *Colias* by the writer show clearly a reduction in interspecific matability as compared to intraspecific matability. Although males are usually ready to mate with females of other species, as far as superficial observations go, only very young females seem inclined to accept males of other species, and even this tendency is uncommon. Although the writer was not able to test the differences in matability which may result from rearing a species on various food plants, it is clear from the writer's experiments that males and females of *C. eurytheme* and *C. philodice* usually pick their own species as mates in the presence of equal numbers of matable males and females of other species. This result does not coincide with Hovanitz's conclusion (1949) from his field observation. However, Fryer (1914) pointed out in *Papilio polytes* Linné that females accept copulation attempts by males most willingly just after their emergence and lose this inclination usually within a day. This phenomenon is true in *Colias* as well as many other species. The typical courting behavior is absent during this receptive period, as Crane (1955) showed in a Trinidad butterfly. In the present experiments, the writer used only females more than one day old. It is possible that *C. eurytheme* or *C. philodice* males often mate with newly emerged females of the opposite species. And it is quite possible that there were many newly emerged females in the field when Hovanitz made his survey. Therefore, these two apparently contrary conclusions could stand together. The writer's experiments in interspecific matings of *C. eurytheme* and *C. philodice* × *C. alexandra* in 1957 (6 interspecific matings were obtained from 14 wild *C. alexandra* males) show that interspecific matings could occur as readily as intraspecific matings, if females were used just after emergence. The extensive mating attempts, using females more than one day old and the same wild *C. alexandra* males used for young females, gave no positive results. However, more experiments are necessary to reveal the real situation in nature. Female butterflies seem to mate at least occasionally more than once in nature, contrary to earlier belief. Therefore, it will be important to discover whether progeny of the first mating, which usually takes place just after emergence of a female, presumably without extensive courtship, make up a large proportion of the next generation.

Although the number of matings obtained was small in most of the inter-

specific matings carried out by the writer, there is no noticeable reduction of fertility of eggs, once females start to lay fertile eggs. Some females which mated with the males of other species laid only infertile eggs, or did not lay any eggs. This also happened in backcrosses of interspecific F_1 hybrids to one of the parental species. Gerould (1943) dissected one F_1 hybrid female (*C. eurytheme* × *C. philodice*) which had mated with the same kind of F_1 hybrid male and did not lay any eggs, and he found one red egg in the vagina. Reddening of an egg is almost certainly proof of fertilization. Although Gerould considered her mate to be sterile, the writer believes that this was a mechanical or physiological failure of egg-laying by the female, since one fertilization unquestionably took place, and it is normal procedure for successive fertilizations to follow after the first fertilized egg is laid. The results of the dissection of 2 females which did not lay eggs in the writer's interspecific matings show the existence of many mature eggs in the ovary, of no red egg in the vagina, and of a good spermatophore in the spermatheca. These facts indicate that no actual fertilization of eggs took place, although the well-formed spermatophore indicates that copulation itself seems to have taken place normally. These phenomena may point to an interspecific physiological barrier, which prevents fertilization or egg laying, similar to the "insemination reaction" which is known in some interspecific hybrids of the genus *Drosophila* (Patterson, 1946). The wild females and F_1 females from wild females which mated with males of the same species usually laid some fertile eggs in the writer's many experiments. A few exceptions of fresh wild females were presumably virgins at the time of their collection.

The examination of hereditary lethality of interspecific hybrids is difficult because the larval death rates by disease are usually very high in confined *Colias*. Yet the writer's results with greenhouse-reared male F_1 hybrids between *C. eurytheme* and *C. interior* show practically no death in their larval stages. One *C. philodice* yellow female, mated with *C. alexandra,* laid only 2 eggs and they did not hatch, although both eggs were fertile. This is probably a result of defect produced by refrigeration of the female while a pupa rather than being due to interspecific inviability.

The slower developmental rate of F_1 hybrid females between *C. eurytheme* and *C. interior* or *C. alexandra,* as compared with the F_1 hybrid males of the same crosses, may have an important meaning in the interspecific isolation in sympatric *Colias* species, if this slow rate also occurs in nature. It is possible that all or many hybrid males emerge during the late summer, while all or many F_1 females emerge early the next summer. If this is true, the production of F_2 individuals would be greatly reduced, even if a large number of F_1 males and females were produced and if they were physiologically and behaviorally capable of producing a good F_2 generation.

Generally speaking, the interspecific matability is low between mountain or northern species (*C. alexandra, C. interior, C. meadii,* and *C. scudderi*) and more widely distributed species (*C. eurytheme* and *C. philodice*), whose distributions are overlapping in many places. Once they have mated, the production of good F_1 individuals, especially males, may be possible in nature as well as in the laboratory. However, the existence of a significant number of F_2 individuals in nature is doubtful, as shown above.

Although it proved to be possible to get the above hybrid matings only in cages, this may be comparable to the situation in which a male finds a newly emerged female in nature. As with a caged female, a newly emerged female in nature can not fly away from an approaching male, since the wings of a newly emerged female are at first too soft for flight. In this light, the relative seasonal isolation between species which the writer

observed in Colorado (Ae, in press) has an important meaning for interspecific isolation. Males of *Colias* emerge earlier than females. Even though they are flying together, there are no, or very few, males of earlier species (*C. philodice*) still flying during the main emergence period of later species (*C. alexandra, C. meadii,* and *C. scudderi;* these differ *inter se* in altitudinal distribution or food plants); and no, or very few, females of later species could appear during the main emergence period of earlier species.

The relationship between *C. eurytheme* and *C. philodice* has been studied more extensively. The low interspecific matability here also seems to be an important factor preventing production of a large hybrid population. Once hybrid matings are accomplished, the production of a large F_1 progeny follows, at least in the laboratory (Gerould, 1943). The reduced fertility and hatchability which appeared in the writer's F_1 brood, P-62-9-7-10, are probably results of deleterious characteristics of the female, which was from an F_3 brood from sib-matings, rather than being due to interspecific inviability, since her sister, which mated with *C. eurytheme* (conspecific) males, had the same reduction in fertility and hatchability.

Gerould (1943) found that F_1 hybrids between *C. eurytheme* and *C. philodice,* by either of the reciprocal crosses, mate together readily. The results of the writer's mating experiments with Brood P-26-9-7-10 coincide with Gerould's data on matability. However, Gerould found 8 out of 9 F_1 matings between *C. eurytheme* females and *C. philodice* males were sterile. Only one out of 4 matings were sterile in the writer's work, and egg fertilities of the above three fertile females were high. Furthermore, one female produced a large number of eggs, although no attempt was made to get the maximum number of eggs from those three females (table 2). The larvae from those eggs grew well without any noticeable breakdown and produced fertile adults. These data indicate a possibility of production of a good F_2 population, in nature, once a mating occurs between two F_1 individuals. However, this possibility in nature can not be very high, because the small population of F_1 hybrids among large populations of *C. eurytheme* and/or *C. philodice* will undoubtedly prevent much crossing between 2 F_1 individuals, since F_1 hybrids mate readily with their parental species.

Orange and yellow ground color expression in hybrids between *C. eurytheme* and *C. interior* or *C. alexandra* seem to be the same as in hybrids between *C. eurytheme* and *C. philodice.* F_1 hybrids show intermediate coloration in all cases. In the backcross to *C. eurytheme* of F_1 hybrids between *C. eurytheme* and *C. interior,* the appearance of a rather large number of typical *C. eurytheme*-like orange color grades and F_1-like color grades may suggest that a rather small number of genes control this hereditary factor (table 3).

The genetic control of orange and yellow ground color in hybrids between *C. eurytheme* and *C. philodice* is still not clear. Gerould (1943) concluded that it is controlled by one pair of genes, with orange incompletely dominant over yellow. However, Hovanitz (1944a) considered that orange coloration is controlled by multiple factors. Remington (1954) showed that it is possible that the orange and yellow wing-colors are controlled by one or at most two pairs of genes having no dominance, on the basis of published and his new unpublished data. The writer obtained many eggs from wild hybrids from the Notre Dame region. The results from rearing some of their progeny show that if the offspring are divided into two groups, orange and pure yellow, the ratio does not differ significantly, by the chi-squared test, from a theoretical 1:1 ratio. The large proportion of yellow individuals clearly shows that a small number of genes control orange-yellow inheritance (table 4). From a crossing between one yellow fe-

TABLE 5. *Color grades of hybrid* C. eurytheme × C. philodice

Parents		C	Color grades of offspring											
			9	8	7	6	5	4	3	2	1	0	T	Total
Female	P–62–9–7–10	8	0	0	0	0	0	0	0	6	4	0	10	10
Male		0	0	0	0	0	1	0	5	6	1	0	13	13
Female	P–62–9–7–10–2	2	0	0	0	0	0	2	0	1	1	0	4	4
Male	P–62–9–7–10–11	2	0	0	1	0	1	2	1	1	0	1	6	7
Female	P–62–9–7–10–9	1	0	0	1	0	0	0	1	1	1	1	4	5
Male	P–62–9–7–10–6	3	0	0	0	0	0	0	0	1	0	1	1	2
Female	P–62–9–7–10–17	1	0	0	0	0	0	1	0	0	0	2	1	3
Male	P–62–9–7–10–5	3	0	0	0	0	0	0	0	0	0	0	0	0
			Total of the above 3 χ^2 (for 3 : 1) = 0.0159									5	16	21
												P ÷ .90		
Female	P–62–9–7–3–4	8	0	0	1	1	0	2	2	4	6	0	10	10
Male	P–62–9–7–10–12	2	1	2	5	6	1	1	0	0	0	0	16	16

80°F, 14 hours light a day.
C—color grades of parents, T—total individuals with some orange coloration.

male from a mixed brood of orange and yellow individuals and a wild *C. philodice* male, the writer obtained an all yellow brood.

The low orange color grades of the F_1 hybrid brood P-26-9-7-10 must have been the result of environmental factors. The *C. eurytheme* which was the mother of this brood was the progeny of 2 successive sib-matings from a wild orange *C. eurytheme* female, and this mother was apparently pure orange genetically (table 5). The rearing room was kept at 80° F and 14 hours light a day. However, the food plants were grown first in the greenhouse in mid-winter without artificial light. The intensity of the 14 hour light illumination in which the larvae were reared was not as strong as sunshine in mid-summer, and therefore there may have been some dilution of the orange coloration. The orange color grades of the F_2 and backcross individuals are scattered extensively, perhaps for the same reason as the F_1 individuals. However, the ratio between orange and yellow in the F_2 generation, 16:5, matches very well a theoretical 3:1 ratio, although the total number is small (table 5).

The data obtained to date are not sufficient to discuss the broad aspects of speciation of the genus *Colias* in North America. However, the following generalizations may be made:

Among the major characters which can be used to separate species, those which have been studied genetically seem to be under simple genetic control. A single pair of genes may control the orange versus yellow ground color difference. Another single pair of genes may control the orange versus yellow hind wing discal spot. Although the evidence is still scant, broad black borders of the wing are probably inherited as dominant over no or very narrow borders in the female.

As far as interspecific hybridization is concerned, there are definite barriers to free hybridization, and the mechanism may be a complicated one. However, these barriers are not absolute in any case well studied. The exchange of genes between different species may not be a very rare event in nature. Hence it is

possible that all North American species are connected through gene exchange by a chain of hybridizations. The relation between *C. eurytheme* and *C. philodice* is complicated, due to extensive natural hybridization. The reason why it is confusing is that the recent sympatry is occurring in a large area, instead of a narrow overlap area, as is more usual and therefore easier to understand. The well surveyed rapid spread of *C. eurytheme* (Hovanitz, 1944b; Gerould, 1946; etc.), aided by rapid clearing of woods in large areas of North America, is undoubtedly responsible for this phenomenon. If this hybrid population increases steadily, with reproductive isolation between *C. eurytheme* and *C. philodice* gradually breaking down, *C. eurytheme* and *C. philodice* will eventually become one species. On the other hand, if the hybrid population decreases, with reproductive isolation between the two species becoming strengthened, *C. eurytheme* and *C. philodice* will become two highly distinctive species. It seems to be most reasonable to regard the two populations as two different species at present; these populations are just on the boundaries of the biological definition of species.

All the new data, presented here and in another paper (in press), give more evidence concerning the close relationship among North American *Colias*. The writer's other paper deals with closely similar developmental rates, prevention of hibernation by controlling only temperature and photoperiod, the relative seasonal isolation among North American *Colias*, and utilization in choice-tests of legumes, blueberry, and willow by *C. scudderi* females for egg-laying and by its larvae for food plants. Not only *C. eurytheme* and *C. philodice*, but all populations of the genus *Colias* seem to be changing more rapidly than many other populations of animals and plants. They appear to be undergoing more rapid speciation than are other, more conservative populations of butterflies. The study of the whole ecology and of the genetics of compatibility and of interspecific differences may be essential to produce a valid classification of these rapid changing populations. Of greater interest, these studies will undoubtedly give a better understanding of evolutionary changing in nature.

Summary

1. The following interspecific hybridizations among 6 species of North American *Colias* were attempted:

a) *C. eurytheme* and *C. philodice* females × *C. alexandra, C. interior,* and *C. scudderi* males
b) *C. eurytheme* females × *C. meadii* males
c) *C. philodice* females × *C. meadii* males
d) *C. interior* female × *C. eurytheme* and *C. philodice* males
e) *C. meadii* female × *C. eurytheme* and *C. philodice* males.
f) *C. eurytheme* × *C. philodice*

Crosses a) and b) were tried more extensively than c), d), e), and f).

2. *C. eurytheme* and *C. philodice* females do not mate with *C. alexandra, C. interior,* and *C. scudderi* males as freely as they mate with the males of their own species. The same is true between *C. eurytheme* females and *C. meadii* males.

3. Interspecific matings between *C. eurytheme* and *C. philodice* seem to take place more readily than the pairings mentioned above. However, interspecific matability between *C. eurytheme* and *C. philodice* is definitely lower than intraspecific matability within each of the two species.

4. The following interspecific matings were obtained:

a) 5 pairs between *C. eurytheme* orange females and *C. alexandra* males
b) 5 pairs between *C. eurytheme* "alba" females and *C. alexandra* males

c) 2 pairs between *C. eurytheme* orange females and *C. philodice* males
d) 1 pair between *C. eurytheme* orange female and *C. interior* male
e) 1 pair between *C. eurytheme* "alba" female and *C. meadii* male
f) 1 pair between *C. philodice* "alba" female and *C. alexandra* male
g) 1 pair between *C. philodice* yellow female and *C. alexandra* male

All of them copulated for a period of more than one hour, and are therefore "successful" in the sense of duration. A copulation of about 5 minutes duration between *C. eurytheme* orange female and *C. scudderi* male was also obtained.

5. Fertile eggs were obtained from the following matings:

a) 4 pairings between *C. eurytheme* "alba" females and *C. alexandra* males
b) 2 pairings between *C. eurytheme* orange females and *C. alexandra* males
c) 2 pairings between *C. eurytheme* orange females and *C. philodice* males
d) 1 pairing between *C. eurytheme* orange female and *C. interior* male
e) 1 pairing between *C. philodice* yellow female and *C. alexandra* male

Two *C. eurytheme* orange females which mated with *C. alexandra* males laid only infertile eggs. The other 5 females did not lay any eggs.

6. The egg fertilities of the above fertile females were generally high.

7. Forty-six males and one female of F_1 hybrids between *C. eurytheme* and *C. interior,* and a total of seven males and one female of F_1 hybrids between *C. eurytheme* and *C. alexandra* were obtained from three broods.

8. F_1 hybrid males of *C. eurytheme* × *C. interior* mated readily with *C. eurytheme* females. Six matings of this kind were obtained. While two of the females laid fertile eggs and one laid only infertile eggs, others did not lay any eggs. The fertilities of eggs which were laid by the above two females were high, and many offspring were obtained.

9. The matabilities and egg fertilities of the females and matabilities of the males of the above mentioned offspring were high.

10. F_1, F_2, and backcross to *C. eurytheme* from *C. eurytheme* × *C. philodice* matings were also obtained.

11. F_1 hybrid males of *C. eurytheme* × *C. interior* and *C. eurytheme* × *C. alexandra* seem to have the same developmental rates as *C. eurytheme* at summer conditions. The developmental rates of their female sibs are very slow. F_1 hybrid female larvae of *C. eurytheme* × *C. alexandra* seem to have an obligatory hibernating stage, and in this they resemble *C. alexandra.*

12. Larvae of hybrids of *C. eurytheme* × *C. interior* stopped feeding mainly during the 3rd instar at conditions of 75° F and 10 hours light a day.

13. The ground (pterine) coloration of the wings of F_1 hybrids of *C. eurytheme* × *C. interior* and of *C. eurytheme* × *C. alexandra* are intermediate between the orange of *C. eurytheme* and the yellow of *C. interior* and *C. alexandra.*

14. Orange and intermediate ground coloration of the wings appeared among progeny from F_1 hybrids of *C. eurytheme* × *C. interior* backcrossed to *C. eurytheme.*

15. The new data support the assumption that orange and yellow ground coloration of the wings of *C. eurytheme* and *C. philodice* are controlled by one or two pairs of genes.

16. Black wing borders of female F_1 hybrid *C. eurytheme* × *C. interior, C. eurytheme* × *C. alexandra,* and female progeny from F_1 hybrid *C. eurytheme* × *C. interior* backcrossed to *C. eurytheme* are as wide as the borders of one parental species, *C. eurytheme,* which has wider

borders in the female than do other three species.

17. Colors of the wing fringes of F_1 hybrid *C. eurytheme* × *C. interior* and *C. eurytheme* × *C. alexandra* somewhat resemble the fringes of *C. interior* and *C. eurytheme,* respectively.

18. The hind wing discal spot is orange on F_1 hybrid *C. eurytheme* × *C. alexandra.*

19. Submarginal dark spots and the dark spot of the costa of the hind wing are present in F_1 hybrid *C. eurytheme* × *C. interior* and *C. eurytheme* × *C. alexandra.*

20. The dusting of fuscous scales on the underside of the wings of F_1 hybrid *C. eurytheme* × *C. alexandra* gives a somewhat grayish-green coloration, as in *C. alexandra.*

21. F_1 hybrids, particularly males, of *C. eurytheme* × *C. interior* and *C. eurytheme* × *C. alexandra* may be produced in nature, and backcrosses of them to their parental species also may occur. However, any significant production of F_2 hybrids in nature is very doubtful.

22. The broad speciation of the genus *Colias* in North America and the taxonomic relationship between *C. eurytheme* and *C. philodice* were discussed.

Acknowledgments

The writer wishes to express his sincere gratitude to Dr. Edward O. Dodson for direction of the research; to other faculty members of the Department of Biology, University of Notre Dame, for their advice; to Dr. Charles L. Remington, Department of Zoology, Yale University, for aid in research and in writing this paper. For their advice and for providing materials, the writer is grateful to the following: Dr. A. E. Brower, Dr. Jane VZ. Brower, Dr. L. P. Brower, Dr. G. B. Fairchild, Dr. J. H. Gerould, Dr. W. Hovanitz, Dr. H. B. Hungerford, Dr. A. B. Klots, Dr. T. Komai, Mr. T. Nomura, Mr. R. W. Pease, Jr., Mr. E. Remington, Dr. M. L. Shubert, Dr. E. G. Voss, and Mr. W. F. Ward.

The writer also wishes to acknowledge the provision of summer facilities by the University of Michigan Biological Station, Cheboygan, Michigan, and The Rocky Mountain Biological Laboratory, Gothic, Colorado.

Literature Cited

Ae, S. A. 1956. Hybrids between *Colias eurytheme* and *C. interior.* Lepid. News, **10**: 9–14 (preliminary report).

——. 1957. Corrections and additions concerning the genetics of *Colias erate poliographus* (Pieridae). Lepid. News, **10**: 143–149.

——. (In press.) Comparative studies of developmental rates, hibernation, and food plants in North American *Colias.* Amer. Midland Nat.

Clarke, C. A., and P. M. Sheppard. 1956. Hand-pairing of butterflies. Lepid. News, **10**: 47–53.

Crane, J. 1955. Imaginal behavior of a Trinidad butterfly *Heliconius erato hydara* Hewitson, with specific reference to the social use of color. Zoologica, **40**: 167–199.

Fryer, J. C. F. 1914. An investigation by pedigree breeding into the polymorphism of *Papilio polytes* Linn. Phil. Trans. Roy. Soc., **204**: 227–254.

Gerould, J. H. 1943. Genetics and seasonal variations of orange wing-color in *Colias* butterflies. Proc. Amer. Phil. Soc., **86**: 405–438.

——. 1946. Hybridization and female albinism in *Colias philodice* and *C. eurytheme.* A New Hampshire survey in 1943 with subsequent data. Ann. Ent. Soc. Amer., **39**: 383–396.

Hovanitz, W. 1943. Hybridization and seasonal segregation in two races of a butterfly occurring together in two localities. Biol. Bull., **85**: 44–51.

——. 1944a. Genetic data on the two races of *Colias chrysotheme* in North America and on a white form occurring in each. Genetics, **29**: 1–30.

——. 1944b. The ecological significance of the color phases of *Colias chrysotheme* in North America. Ecology, **25**: 45–60.

——. 1948. Ecological segregation of interfertile species of *Colias.* Ecology, **29**: 461–469.

——. 1949. Interspecific matings between *Colias eurytheme* and *Colias philodice* in wild populations. EVOLUTION, **3**: 170–173.

KOMAI, T., AND A. S. AE. 1953. Genetic studies of the pierid butterfly, *Colias hyale poliographus*. Genetics, **39**: 65–72.

PATTERSON, J. H. 1946. A new type of isolating mechanism. Proc. Nat. Acad. Sci. (Wash.), **32**: 202–208.

REMINGTON, C. L. 1954. The genetics of *Colias* (Lepidoptera). Advances in Genetics, **6**: 403–450.

——. 1955. The inheritances of hind wing discal spot color in *Colias philodice*. Lepid. News, **8**: 163–166.

STEINHAUS, E. A. 1949. Principles of insect pathology: 477–485.

TALBOT, G. 1935. Pieridae III. Lepidopterorum Catalogus, **66**: 408–492.

GEOGRAPHICAL DIFFERENTIATION OF INVERSION SYSTEMS IN *DROSOPHILA SUBOBSCURA*

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Received April 15, 1958

There is no group of organisms in which the analysis of structural changes in chromosome organization has been carried further than in the Diptera. In several genera belonging to different families of this order, repatterning of the chromosomes was shown to be due, in large measure, to inversions (Patterson and Stone, 1952; Stone, 1955; Philip, 1942; Beermann, 1953, 1955; Acton, 1957; Rothfels, 1956). These rearrangements are involved in numerous cases of subspecific and specific differentiation and thus constitute a progressive factor in microevolution. However, within the limits of a given population, inversions may fulfill a function, which is essentially conservative. Numerous populations, belonging to different species of Drosophila, Chironomus, Sciara or Simulium are polymorphic for several structural types of their chromosomes. Recombination between gene sequences, which are inverted relative to each other is effectively prevented in these populations and the integrity of the gene strings is thus guaranteed. A series of experimental studies initiated by Wright and Dobzhansky (1946) and Dobzhansky (1948) has shown how much the coexistence of several intact gene orders contributes to the viabilities of these polymorphic populations of flies. Since the efficiency of inversion systems in preserving coadapted gene series has been given such convincing proof it is the more intriguing to find that this mechanism is utilized to widely different extents by different species.

In a recent review Stone (1955) has contrasted the *virilis* group of Drosophila, many of whose species depend on comprehensive structural polymorphism, with the *repleta* group in which this phenomenon is of much more limited occurrence. These different types of population structure appear in no way correlated with the prevalent modes of speciation, for Stone and his collaborators were able to demonstrate the contributions of inversions to the distinctive chromosomal architecture of almost any species in both groups. The problem gains additional interest by the curious fact that cosmopolites like *Drosophila virilis, D. repleta* and *D. hydei* irrespective of their affiliation to one or the other group are extremely restricted in their utilization of structural polymorphism.

Our comprehension of the laws governing the balance between diversity and uniformity of chromosomal types in populations may be expected to gain from the study of those species whose geographic races differ in the extent of their polymorphism. Da Cunha, Burla and Dobzhansky (1950) and da Cunha and Dobzhansky (1954) have pointed out that in *Drosophila willistoni* inversion polymorphism is richest in the centre of the distribution area falling off towards the margins (Townsend, 1952). Chromosomal diversity in this species is, moreover, positively correlated with the ecological diversification and the luxuriance of the vegetation in the area inhabited by any particular geographic race. Recently, Dobzhansky (1957) has demonstrated that the polymorphism of numerous populations of *D. willistoni* in the West Indies

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and in Central America is in full accordance with the expectations based on these rules. Not only does structural heterozygosity decrease with the distance from the South American continent; within the archipelagos its extent is also clearly correlated with the size of the islands. Throughout these comprehensive studies, the average number of inversion per individual served as an index of the structural diversity in a given population.

The present authors in several previous studies (Stumm-Zollinger, 1953; Goldschmidt, 1956, 1958) attempted to compare populations of *Drosophila subobscura* mainly by means of this index. The results failed to indicate a correlation of the average number of inversions with the diversity of the vegetation in the different biotopes in western Europe (Stumm-Zollinger, 1953). Nor could the marginal populations of Israel (Goldschmidt, 1956) be shown to differ by this standard from the majority of the races of Switzerland, which lies well in the centre of the species area. Each of us was therefore inclined to assume that the "working hypothesis" of da Cunha and Dobzhansky (1954) based on their experience with a neotropical species did not apply to our palaearctic fly. There were, however, several reasons for doubting the validity of the number of inversions per individual as the sole index of structural polymorphism in our species. While many of the inversions in *willistoni* are small and independent the inversions of *D. subobscura* are widely divergent in size (cf. table 2). They also show a pronounced tendency to overlapping in complex heterozygotes. This tendency is particularly manifest in the Israel populations of *D. subobscura* where most of the prevalent configurations are complex. It was felt that mere counting of the number of inversions could not possibly yield a true measure of the chromosome lengths isolated in the average individual of our populations. A similar problem has been solved with much success by Carson (1955a, b) working with *D. robusta*. His "index of free recombination" estimates the average lengths of euchromatin which are devoid of inversions and therefore potentially free to undergo crossing-over. Carson, by showing this index to increase from 65% in the centre of the distribution of *D. robusta* to 85% in the marginal areas (and even to 100% in the case of one population) furnished a beautiful corroboration of the hypothesis of da Cunha and Dobzhansky (1954).

In the present communication Carson's "index of free recombination" is applied to the populations of *D. subobscura* which have so far been analyzed, and the information derived from different measures of the inversion load is evaluated.

Methods

Of the 16 populations dealt with here, no. 1–13 have been described by one of us (Stumm-Zollinger, 1953) and no. 14 and 15 by the other (Goldschmidt, 1956, 1958). Population 16 is first reported here.

Table 1 briefly lists some geographical and biotic data for each population. (A fuller description of localities 1–13 may be found in Stumm-Zollinger, 1953.)

Our calculations of the relative inversion lengths are based on the map of Mainx, Koske, and Smital (1953). Professor Mainx has kindly informed us of a modification of this map adopted in his laboratory (the addition of section 101 at the left end of the U-chromosome) and this has been taken into account. The exact localization of the inversions in the present species is of particular interest in view of the pronounced non-randomness of the break-points (Stumm-Zollinger, 1953; Kunze-Mühl and Sperlich, 1955; Goldschmidt, 1956) and there has been much collaboration in this field between the various laboratories working on *D. subobscura*. The present authors were therefore able to agree on the localization of most of the inversions which are common to Europe and Israel. In a few instances where our respective estimates differed (never by more than 0.4%), we

TABLE 1. *Geographic and biotic characterization of the 16 localities investigated*

1. ***Biaz*** France, Landes, 6 kms south of Mimizan. Extremely monotonous vegetation, consisting chiefly of *Pinus maritima,* with undergrowth of *Ericaceae* and *Genista.* At forest edge a few oaks, sycamores (*Platanus*) and bramble hedges (*Rubus*).

2. ***Tellay*** France, Bretagne, 35 kms south of Rennes. Mixed forest of deciduous trees with some pines (*Pinus*) and firs (*Abies*) and rich hedges.

3. ***Lisbon*** Portugal. Left bank of Tejo River, 11 kms north-east of Lisbon. Sandy, dry soil with pure forest of *Quercus suber;* dry grass, thistles (*Carduus*). At a distance of 800 ms some *Quercus ilex.*

4. ***Garonne*** France. Bank of Garonne River, 30 kms south of Toulouse. *Populus* and *Alnus* growing along river bed, further uphill dense deciduous forest, farms in neighborhood.

5. ***Bex*** Switzerland, Lower Wallis. Mild climate, rich vegetation. Trapping in beech forest (*Fagus sylvatica*).

6. ***Thun*** Switzerland. Slope with southern exposure, 300 ms from shore of Lake Thun. Mixed forest of oak, lime (*Tilia*) and maple (*Acer*). Orchards in neighborhood.

7. ***Chateauneuf sur Loire*** France. Chenaille, 20 kms east of Orleans. Mixed forest with clearing. Oaks, pine, birch (*Betula*), chestnut (*Castanea*), poplar (*Populus*), willow (*Salix*), elder (*Sambucus*), bramble (*Rubus*).

8. ***Eglisau*** Switzerland, left bank of Rhine. Humid river bank densely grown with horse chestnut (*Aesculus hippocastaneum*). Mixed deciduous forest 300 ms farther down river.

9. ***Fetan*** Switzerland. Alpine locality near tree line, in the belt of larches (*Larix europaea*) and pine (*Pinus cembra*), shrubs of *Berberis vulgaris,* alpine meadows. Short vegetation period.

10. ***Vitznau*** Switzerland. Lake of four Cantons, mild and humid climate. Beech forest (*Fagus sylvatica*), shrubs (*Rubus* and *Cornus*), orchards.

11. ***Küsnacht*** Switzerland. Northeastern shore of Lake Zürich. Meagre, acid soil, beech forest with shrubs (*Rubus* spp. *Cornus sanguinea, Sambucus nigra*). Orchards in neighborhood.

12. ***Oberdorf*** Switzerland, north-east of Solothurn. Beech forest with a few oaks. Sparse undergrowth of strawberry (*Fragaria vesca*), bramble (*Rubus*) and *Cornus sanguinea.*

13. ***Braunwald*** Switzerland. Southeastern slope of the Lin valley, in the belt of spruce (*Picea excelsa*) and maple (*Acer*) just above the belt of beech (*Fagus sylvatica*) and fir (*Abies alba*). Blueberry shrubs (*Vaccinium*). Moist and cool, no fruit cultivation.

TABLE 1.—(*Continued*)

14. ***Qiryat 'Anavim*** Israel. Agricultural settlement and small neighboring oak wood (*Aquabella*) in Judean Hills. Mediterranean vegetation of shrubs and perennial herbs. Residual maquis (association of *Quercus calliprinos* and *Pistacia palaestina*). Pine trees (*Pinus halepensis*). Vegetable gardens and orchards (almonds, plums, peaches, vine).

15. ***Eilon*** Israel. Settlement on northwestern slope of Upper Galilee. Mediterranean vegetation of maquis (*Quercus calliprinos-Pistacia palaestina*—association, containing also *Quercus infectoria*). Carob trees (*Ceratonia siliqua*), orchards, vineyards.

16. ***Oranim*** Israel. Settlement on southern Foothills of Lower Galilee. Mediterranean vegetation of shrubs and perennial herbs. Remnants of Tabor oak park forest (*Quercus ithaburensis, Q. calliprinos, Styrax officinalis*). Carob (*Ceratonia siliqua*) and pine (*Pinus halepensis*) plantations.

chose the mean between them. Eva and E_{12} whose identity could not be definitely ascertained, are treated as two different inversions. Inversions of the O-chromosomes are particularly diversified and many of them are endemic and rare in their respective localities. We have therefore refrained from assuming identity of O-arrangements found in different countries, even when their break-points appeared similar. Solely for O_{3+4} and for O_7, which are widely distributed have we agreed on a common value, whereas for all other O-inversions the values for Europe were determined by Stumm-Zollinger and those for Israel by Goldschmidt. We believe that the overall result of our calculation cannot be much affected by any small discrepancies between our respective localization of some of the rarer inversions.

The relative inversion lengths underlying our calculations are summarized in table 2, which we hope may serve as a frame of reference for further analyses of *D. subobscura* from other areas.

The average index of free recombination for each population was based on the salivary chromosome analysis of female larvae, each of which was the daughter of a different female caught inseminated

TABLE 2. *Relative lengths of chromosomes and inversions of* D. subobscura

Chromosome	Percentage of total euchromatin	Simple and compound inversions	Percentage of total euchromatin
A	16.2	A_1 (Attila)	5.3
		A_2 (Aldo)	4.1
J	19.8	J_1 (Ingrid)	3.0
		J_{3+4}	7.6
U	18.6	U_1 (Urs)	5.7
		U_8 (Ursula)	4.8
		U_4	4.9
		U_2 (Umberto)	6.4
		U_3 (Undine)	5.2
		U_7	6.8
		$U_3 + U_2$	7.0
		$U_3 + U_7$	7.1
		$U_7 + U_4$	12.1
		$U_2 + U_3 + U_5$	8.3
		U_9	1.9
E	20.0	$E_1 + E_2$ (Edda & Eleonore)	5.6
		E_9 (Ernst)	9.1
		E_8 (Emanuel)	7.0
		Eva	6.5
		E_{12}	4.2
		$E_1 + E_2 + E_8$ (Edda, Eleonore & Emanuel)	9.3
		$E_1 + E_2 + E_9 + E_{12} + E_8$	9.3
		$E_{12} + E_3$	4.7
O	24.4	$O_3 + O_4$ (Orson & Ottilie)	7.4
		O_8 (Olga)	6.5
		Orest	2.3
		O_{15} (Othmar)	7.1
		O_7 (Oskar)	7.4
		O_{12} (Ortega)	3.3
		Othello	3.0
		O_1 (Oswald)	3.6
		O_6 (Ossian)	5.0
		Ottokar	3.8
		O_2	3.9
		O_{19}	5.1
		O_{20}	5.6
		$O_2 + O_5$	5.3
		$O_2 + O_{18}$	4.4
		$O_2 + O_{17}$	7.5
		O_{21} (= O_1 ?)	3.8
		O_{24}	5.4

Total 100.0.

in the wild. Following Carson (1955a) the males in which crossing-over is absent regardless of their chromosome structure, were not included in the estimate.

RESULTS

In table 3 the populations are arranged according to their index of free recombination in ascending order. The table also lists the percentages of completely homozygous females, the average inversion frequencies and the limits of inversion incidence in males and females and the number of inversion types found at each locality.

The extreme values of the index of free

TABLE 3. *Polymorphism of 16 populations of* D. subobscura *measured by a series of indices*

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Locality	Country	Height above sea-level	Average annual rainfall mm	Index of free recomb. (Mean ± S.E.)	Per cent homozygous females (Ratio ± S.E.)	Average no. inversions per ♀	No. ♀♀	Average no. inversions per ♂	No. ♂♂	No. inversions limits ♂♂ and ♀♀	No. of inversion types found
1. Biaz	France	0	800–1000	79.4 ± 1.2	1.6 ± 1.6	4.8	62	4.0	26	0–11	18
2. Tellay	France	100	750	82.1 ± 1.2	0	4.6	40	4.1	20	0–9	18
3. Lisbon	Portug.	0	500–600	82.5 ± 1.6	6.1 ± 4.2	4.2	33	3.4	11	0–10	15
4. Garonne	France	150	600–800	83.3 ± 1.0	1.9 ± 1.9	4.4	54	4.0	14	0–9	18
5. Bex	Switz.	400	1120	84.1 ± 1.2	4.2 ± 2.9	3.3	48	2.8	36	0–9	15
6. Thun	Switz.	560	920	85.0 ± 1.1	5.1 ± 2.9	3.8	59	2.9	24	0–11	16
7. Chateauneuf	France	100	500–750	85.0 ± 0.9	4.5 ± 2.7	3.4	66	3.5	33	0–9	17
8. Eglisau	Switz.	400	970	85.2 ± 0.7	5.5 ± 1.9	3.2	145	2.5	68	0–9	21
9. Fetan	Switz.	1750	710	85.3 ± 1.1	8.3 ± 3.6	3.3	60	2.3	22	0–8	15
10. Vitznau	Switz.	430	1650	86.0 ± 1.2	2.3 ± 2.3	3.7	44	2.6	27	0–8	14
11. Küsnacht	Switz.	400	1140	86.6 ± 1.6	4.4 ± 1.6	3.3	159	2.4	83	0–10	19
12. Oberdorf	Switz.	650	1270	87.5 ± 0.8	4.8 ± 2.3	3.2	84	2.4	19	0–8	17
13. Braunwald	Switz.	1280	1870	87.8 ± 0.9	9.2 ± 2.8	2.4	76	2.5	21	0–7	16
14. Qiryat 'Anavim	Israel	625	653	88.8 ± 0.6	16.4 ± 2.8	3.5	171	3.3	76	0–11	26
15. Eilon	Israel	325	812	89.6 ± 0.8	18.5 ± 4.3	3.9	81	3.1	37	0–10	21
16. Oranim	Israel	100	665	89.8 ± 1.3	9.7 ± 5.3	3.3	31	4.2	69	0–12	21
						Total	1213	Total	586		

TABLE 4. *Significance of differences between Indices of Free Recombination in 16 populations of* D. subobscura

	Biaz	Tellay	Lisbon	Garonne	Bex	Thun	Chateauneuf	Eglisau	Fetan	Vitznau	Küsnacht	Oberdorf	Braunwald	Qiryat 'Anavim	Eilon	Oranim
Biaz																
Tellay	−															
Lisbon	−	−														
Garonne	+	−	−													
Bex	++	−	−	−												
Thun	++	−	−	−	−											
Chateauneuf	+++	+	−	−	−	−										
Eglisau	+++	+	−	−	−	−	−									
Fetan	+++	−	−	−	−	−	−	−								
Vitznau	+++	+	−	−	−	−	−	−	−							
Küsnacht	+++	+++	++	++	+	−	−	−	−	−						
Oberdorf	+++	+++	++	++	+	−	+	+	−	−	−					
Braunwald	+++	+++	++	++	+	+	+	+	−	−	−	−				
Qiryat 'Anavim	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	−	−			
Eilon	+++	+++	+++	+++	+++	+++	+++	+++	++	+	++	−	−	−		
Oranim	+++	+++	+++	+++	++	++	++	++	+	+	+	−	−	−	−	

\+ p = <0.05
++ p = <0.01
+++ p = <0.001
− p = >0.05

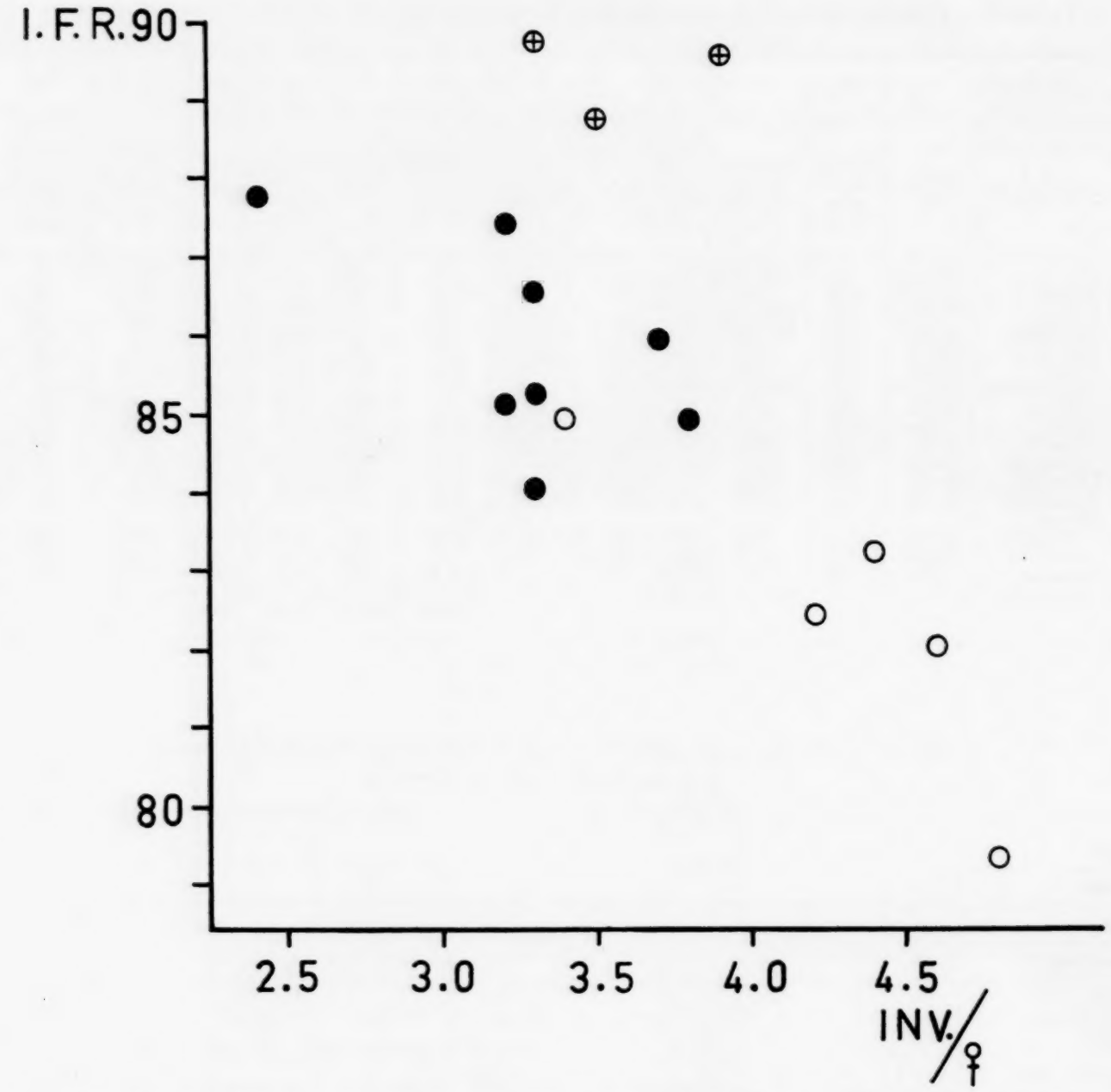

FIG. 1. Index of free recombination in sixteen populations of *D. subobscura* plotted against average inversion frequency per female.

○ French and Portuguese populations
● Swiss populations
⊕ Israel populations

recombination (I.F.R.) for our populations differ by barely more than ten per cent. In spite of this comparatively narrow range of variation the index allows of a fair classification of the localities according to geographic areas. The populations of France and Portugal are characterized by the lowest Index and show little overlap with those of Switzerland. All three populations of Israel, which is presumably close to the Southern boundary of the species area (cf. Buzzati-Traverso and Scossiroli, 1955) rank at the bottom of the list with the highest amount of free crossing-over. They differ significantly from all but two of the Swiss populations (cf. table 4 for significance of differences). A glance at table 3 (column 7) shows that the average inversion frequency in females fails to differentiate the Israel populations from the European ones.

In figure 1 the I.F.R. is plotted against the average inversion load. These two measures of polymorphism show a high negative correlation within the area of

Western and Central Europe. The coefficient of correlation for all thirteen European populations is -0.88 ($t = 6.03$). It is in no way surprising that the two indices convey much the same information when it is remembered that the European populations share many of the main structural arrangements, differing only in the frequencies of some of them. Stumm-Zollinger (1953) has shown that the mean inversion incidence at the French and Swiss localities bears no obvious relationship to the bioclimatic characteristics of the environment. In view of the considerable negative correlation between the average inversion load and the I.F.R. it is not unexpected that the latter index, too, varies to all appearances independently of annual rainfall, diversification of vegetation and height above sea level.

Carson (1955b) discussing the European populations of *D. subobscura* tends to interpret the lower inversion incidence in Switzerland as compared with France as a result of the quasi-marginal conditions prevailing in the Alps. The data of table 3 present little evidence for a connection between altitude and inversion concentration. A depression of the I.F.R. in the lowland populations of Switzerland by genetic flow from the alpine areas would seem more plausible if the polymorphism showed any clinal tendency towards minima in the mountainous regions. Yet, we are aware that the pace and range of such genetic flow cannot be estimated without a knowledge of population sizes and migration rates in our species. As pointed out by Stumm-Zollinger (1953), *D. subobscura* reveals only very little tendency to microdifferentiation, geographical races of fairly uniform type inhabiting wide areas including many different biotopes.[2]

The three populations of the Middle Eastern race are closely similar to one another, but differ in numerous respects from the European ones. They occupy a separate position on the correlation graph (fig. 1). When they are included in the calculation the coefficient of correlation for the sixteen localities is reduced to -0.68 ($t = 3.45$). This drop in correlation is a neat reflection of the fact that inversion systems in the Near East differ for each chromosome from those of France and Switzerland. Most of the common structural arrangements of Europe are replaced in Israel by other chromosome orders.

While the I.F.R. proves, in this instance, to be a more delicate indicator of racial differentiation than the average inversion incidence, it is far from conveying the whole information. Thus, it is instructive to compare the inversion loads of males at the various localities (table 3, column 9). Measured by this standard the Israel populations would be classed among the French and Portuguese. This change of "rank" is due to the fact that X-chromosome polymorphism plays a minor role in Israel but is very important in Europe, where males contain therefore fewer inversions than females. The European and the Near Eastern populations also differ in the role played by the O-chromosome. This element, the longest of the autosomes, is very highly polymorphic in all our Western populations and only rarely heterozygous in the East. Since polymorphism involves all five chromosomes to a more or less equal extent in Europe, a fly in this area has a small chance of being completely homozygous (table 3, column 6). In Israel, where heterozygosity is mainly concentrated in three of the five elements, there is a higher percentage of completely homozygous individuals. Owing to the

[2] Of the three localities in Israel, Oranim lies about 100 kms North of Quiryat 'Anavim and Eilon is situated another 40 kms further north. Nevertheless, significant differences between the three populations concern only two of the commoner inversions: The concentration of O_7 at Oranim (13.0%) differed significantly ($p = 0.05 - 0.02$) from that of Q. 'A. (6.1%) and of Eilon (4.2%), while E_{12} at Eilon amounted to only 35.6% and was thus significantly ($p = 0.05 - 0.02$) rarer than at Q. 'A. (47.4%) or at Oranim (50.0%).

complexity of structural heterozygotes in Israel the limits of inversion incidence are as wide as, or wider than in Europe (table 3, column 11). The most heterozygous individual of *D. subobscura* described so far was a male from Oranim carrying twelve inversions. Finally, column 12 of table 3 indicates that the variety of inversion types is probably richer in the Eastern populations at the margin of the deserts than in the heart of Europe. Altogether, 29 different inversions [3] have been identified in the populations from Israel, while the much larger territory investigated in Europe revealed only 23.

Discussion

The index of free recombination differs significantly, though not very widely, in *D. subobscura* inhabiting different territories. This index conforms to the "working hypothesis" of da Cunha and Dobzhansky (1954) in being somewhat higher in the marginal populations of Israel than in Central and Western Europe. While it is a remarkable fact that both *D. robusta* and *D. subobscura* exhibit certain parallels with the pattern of geographic variation described for *D. willistoni,* the biological interpretation of this variation requires much caution. The I.F.R. is based on measurements of the chromosome lengths covered by simple or compound inversions. Carson's (1953) analysis of the intra- and inter-chromosomal effect of inversions on crossing over has shown afresh that these interactions are complex and can on no account be predicted from the relative lengths of the inverted sections. Levine (1956) has demonstrated how widely inversions may differ in the amount of double crossing over, which they permit. Dobzhansky and Epling (1948) found that compound inversions in the third chromosome of *D. pseudoobscura* virtually suppress recombination over the whole of the element and that no crossing over whatsoever is detectable in the inverted sections. If the integrity of the gene strings in coadapted orders differing by simple inversions is perpetually threatened by double crossing over, this danger is presumably minimized in the compound heterozygotes of the *D. subobscura* in the Near East, where most of the common orders differ by two to five inversions. (Crossing over in this race is blocked on the average over a somewhat smaller fraction of the total genome than in its European relatives; but it appears to be blocked more efficiently.)

Comparing our populations by a series of different indices we find that none can be dispensed with in a full description of racial differentiation. The most significant difference between the European and the Eastern populations may lie in the number of elements involved in polymorphism. The virtual restriction of heterozygosity to three of the five chromosomes (J, U, E) which characterizes the race of the Middle East is most clearly reflected in the high number of homozygous individuals. The complexity of structural heterozygotes in this race finds expression in the wide limits of inversion incidence. If the number of structural types represented in each area were chosen as a yard stick of polymorphism the Eastern populations would rank as the most diversified.

No single method of counting or measuring inversions suffices to characterize or classify polymorphic systems of widely different geographic races. The structural diversity of a population will be described as "richer" or "poorer" depending on the standard which is applied. Nobody can be more aware of these difficulties than da Cunha and Dobzhansky (1954), and Dobzhansky (1957). In their extensive survey of *D. willistoni* they detected a general tendency of both variety of inversions and limits of heterozygosity to decline with the average inversion load. But these correlations were

[3] In addition to the 28 inversions listed by Goldschmidt (1958) a new inversion O_{24} was found at Eilon and localized as follows: 79 B 3–4—85A.

by no means strict. Thus 25 types of inversions were found both at Carolina Maranhão and at Montez Claros, Minas Gerais, but the average frequency of inversions in females of the first locality amounted to 8.65 ± 0.58 while at the second it was only 3.54 ± 0.28. At Montez Claros a fly may bear up to ten inversions, in Costa Rica, where the average inversion load is similar (3.25 ± 0.85) the maximum was only four. These investigators also discovered some races in which polymorphism is restricted to four or three of the five chromosome arms, but this phenomenon was not accompanied with increased complexity in the remaining elements. Referring to insects other than Diptera, White (1957) has cited several instances constituting apparent exceptions to Dobzhansky's rule. His discussion, as well as the present one actually tends to confirm the value of this working hypothesis as an approach to the classification of the confusing variety of structurally polymorphic populations. It is hoped that future work on *D. subobscura* from other areas will furnish a more complete picture of its racial differentiation.

Summary

1. The structural heterozygosity in sixteen populations of *D. subobscura* from Central and Western Europe and from Israel is compared by the index of free recombination proposed by Carson (1955). This index is significantly lower in France and Portugal than in most Swiss populations. It is higher in Israel than in the populations of Europe.

2. The index of free recombination is compared with other measures of polymorphism and some of their biological implications are discussed.

Acknowledgments

We are greatly obliged to the staff of the teacher's seminary at Oranim and to the members of the settlement Eilon for their help and hospitality during collection trips to their areas. The assistance of Dr. R. Falk and of Mrs. Ada Lederman-Klein in trapping flies is gratefully acknowledged. The slides for analysis of populations 15 and 16 were skilfully prepared by Miss Sarah Rapaport. Finally we should like to thank Dr. Naomi Feinbrun for her kind advice with the geobotanical description of the Israel localities and Dr. H. Burla for reading and criticizing the manuscript.

Literature Cited

Acton, A. B. 1957. Chromosome inversions in natural populations of *Chironomus tentans*. J. Genet., **55**: 61–94.

Beermann, W. 1953. Chromosomenpolymorphismus und Bastardierung zweier *Chironomus*-Arten. Zool. Anz. Suppl., **17**: 290–295.

——. 1955. Cytologische Analyse eines *Camptochironomus*-Artbastards. I. Kreuzungsergebnisse und die Evolution des Karyotypus. Chromosoma, **7**: 198–295.

Buzzati-Traverso, A., and Scossiroli, R. E. 1955. The "Obscura group" of the genus Drosophila. Adv. Genetics, **7**: 47–92.

Carson, H. L. 1953. The effects of inversions on crossing over in *Drosophila robusta*. Genetics, **38**: 168–186.

——. 1955a. Variation in genetic recombination in natural populations. J. Comp. Cell. Phys., 45 suppl., **2**: 221–236.

——. 1955b. The genetic characteristics of marginal populations of Drosophila. Cold Spring Harbor Symp. Quant. Biol., **XX**: 276–287.

da Cunha, A. B., Burla, H., and Dobzhansky, Th. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution, **4**: 212–235.

——, and Dobzhansky, Th. 1954. A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. Evolution, **7**: 119–134.

Dobzhansky, Th. 1948. Genetics of natural populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographic regions. Genetics, **33**: 588–602.

——. 1957. Genetics of natural populations. XXVI. Chromosomal variability in Island and continental populations of *Drosophila willistoni* from Central America and the West Indies. Evolution, **11**: 280–293.

——, and Epling, C. 1948. The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. Nat. Ac. Sci., **34**: 137–141.

GOLDSCHMIDT, E. 1956. Chromosomal polymorphism in a population of *Drosophila subobscura* from Israel. J. Genet., **54**: 474–496.

——. 1958. Polymorphism and coadaptation in natural populations of *Drosophila subobscura*. Proc. Tenth Int. Congr. Ent. Montreal, **2**: 821–828.

KUNZE-MÜHL, E., AND SPERLICH, D. 1955. Inversionen und chromosomale Strukturtypen bei *Drosophila subobscura* Coll. Zeitschr. ind, Abst. Vererb., **87**: 65–84.

LEVINE, R. P. 1956. Crossing over and inversions in coadapted systems. Am. Nat., **90**: 41–45.

MAINX, F., KOSKE, T., AND SMITAL, E. 1953. Untersuchungen über die chromosomale Struktur Europäischer Vertreter der *Drosophila obscura* Gruppe. Zeitschr. ind. Abst. Vererb., **85**: 354–372.

PATTERSON, J. T., AND STONE, W. S. 1952. Evolution in the genus *Drosophila*. Macmillan, N. Y.

PHILIP, U. 1942. An analysis of chromosomal polymorphism in two species of Chironomus. J. Genet., **44**: 129–142.

ROTHFELS, K. H. 1956. Black flies: siblings, sex, and species grouping. J. Hered., **47**: 113–122.

STONE, W. S. 1955. Genetic and chromosomal variability in Drosophila. Cold Spring Harbor Symp. Quant. Biol., **XX**: 256–270.

STUMM-ZOLLINGER, E. 1953. Vergleichende Untersuchungen über die Inversionshäufigkeit bei *Drosophila subobscura* in Populationen der Schweiz und Südwesteuropas. Zeitschr. ind. Abst. Vererb., **85**: 382–407.

TOWNSEND, J. I., JR. 1952. Genetics of marginal populations of *Drosophila willistoni*. EVOLUTION, **6**: 428–442.

WHITE, M. J. D. 1957. Some general problems of chromosomal evolution and speciation in animals. Surv. Biol. Progr., **3**: 109–147.

WRIGHT, S., AND DOBZHANSKY, TH. 1946. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics, **31**: 125–156.

ZOHARY, M. 1955. Geobotany. Hashomer haza'ir Press, Merhaviah. (Hebrew.)

THE PELYCOSAUR TYMPANUM AND EARLY EVOLUTION OF THE MIDDLE EAR

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Received April 15, 1958

INTRODUCTION

Two groups of primitive reptiles of early Permian age, the Pelycosauria and Captorhinomorpha, are characterized in part by a massive, firmly attached stapes, and by a flat or slightly convex posterior skull margin. The characteristics of the middle ear region contrast strongly with conditions in modern reptiles, in which the stapes is very slender, and the concave posterior margin of the skull supports a superficial tympanum that functions in detection of air-borne sound. The middle ear region of the fossil amphibians, ancestors of reptiles, also probably functioned to detect air-borne sound by means of a tympanum, which presumably was housed in the otic notch on the dorso-posterior margin of the skull. The tympanum resembled that of modern reptiles in its superficial position, but the stapes was primitively more massive and more firmly attached. The peculiarities of the pelycosaur middle ear have led to conflicting interpretations of its function and of its position in the general evolution of the tetrapod ear. A radical approach to the matter is that of Tumarkin (1955), who denies that the pelycosaurs and captorhinomorphs were sensitive to air-borne sounds. Watson (1948 *et seq.*), while he accepts the probability that these forms had a middle ear cavity and could hear, believes that the tympanum was lost upon closure of the otic notch. A crucial question in the functional interpretation of the pelycosaur middle ear is the size of membrane necessary to actuate a stapes as massive as that found in some fossil forms. The experimental part of the present study is an attempt to provide an answer to this aspect of the problem. Another difficulty in interpreting the pelycosaur middle ear is placement of a tympanum. Watson's claim that a tympanum could not have been supported superficially on the back of the pelycosaur skull is countered by Romer and Price (1940), who suggest simply that it was placed in a deep position, where it lay behind the quadrate and terminal to the stapes as it does in modern reptiles. Morphological relationships of the pelycosaur stapes suggest a different mode of tympanum support, which is elucidated in the body of this paper. The course of early evolution of the tetrapod middle ear is interpreted in the light of function and support of the tympanum in the pelycosaurs.

MORPHOLOGY AND FUNCTION OF LOWER TETRAPOD MIDDLE EARS

The middle ear chamber of most modern reptiles is characteristically developed as a lateral outpouching of the pharynx, lying behind the quadrate and pterygoid bones and below the paroccipital process (fig. 1, A). It is roughly cylindrical in shape, transversely oriented, and is lined with a membrane of mucous epithelium to which the term tympanic membrane is often applied. Laterally this membrane reaches nearly to the surface of the head, where it joins a circular or oval area of skin to form the tympanum, or ear-drum, which thus serves to close the outer end of the cylinder. Connection between the skin and the underlying epithelium is rather loose; although the epithelium is exceedingly delicate, it is not damaged when the skin is peeled away in dissection of preserved specimens. The tympanum is attached to the back of the

quadrate in adult crocodilians, turtles and most lizards. In the lizard the drum is also attached inferiorly to the articular bone (retroarticular process) and may receive additional support from other bones in the vicinity. Although Oelrich (1956) describes most of the connection of the ear-drum to surrounding bone as cutaneous, when the skin is peeled away the epithelial lining remains attached to bone in essentially the same places. Functionally the tympanum may be considered as a single unit, with bony connections as outlined above. The stapes, a slender rod of cartilage and bone, closes the fenestra ovalis in the lateral wall of the otic capsule by means of its slightly expanded proximal end, the footplate. From the fenestra ovalis it passes laterally across the tympanic cavity to attach eccentrically to the tympanum. In the adult reptile there are often two short and slender ligaments near the distal end of the stapes, a dorsal process running up to the tip of the paroccipital process, and an internal process running forward and downward to the quadrate. The part of the shaft that lies distal to dorsal and internal processes, and attaches to the tympanum, is called the tympanic process.

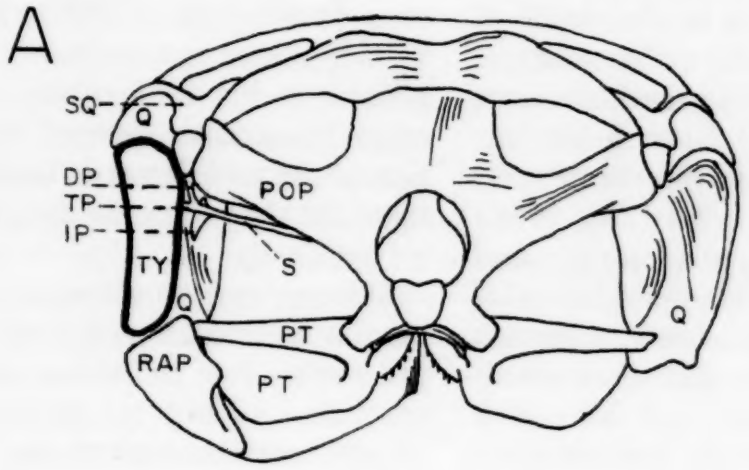
In all modern reptiles with a well-developed tympanum the middle ear functions to detect air-borne sound; sound waves impinge upon the tympanum and the resulting vibration is transmitted along the stapes to the inner ear. In such forms the quadrate and stapes appear to be specifically adapted to these functions. The quadrate is deeply concave posteriorly (dorso-posteriorly in the crocodilians) for support of the ear-drum; in lizards there is a pronounced ridge on the C-shaped lateral margin of the quadrate and on the latero-dorsal margin of the articular, to which the drum attaches. The stapes is extremely slender, and its attachments, except the tympanic process, are much reduced in most living forms.

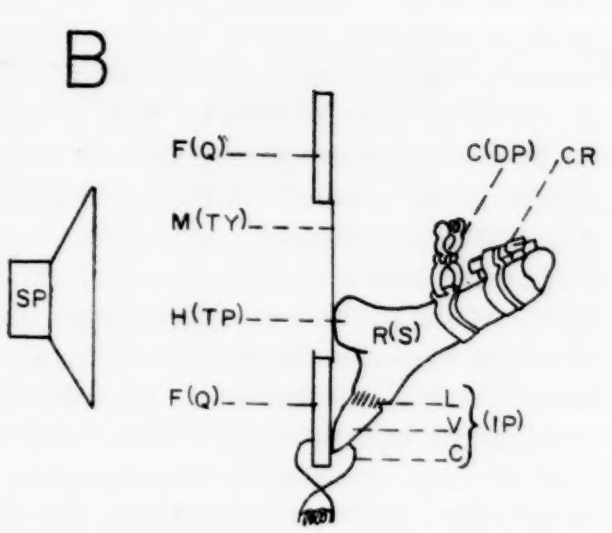
In primitive tetrapods generally, the stapes tends to be of much more massive build than in modern reptiles, this tendency being carried to an extreme in large pelycosaurs like *Ophiacodon* and *Dimetrodon* (fig. 1, C). The stapes of captorhinomorph reptiles (and probably of smaller pelycosaurs) is more massive in build than the stapes of modern reptiles, especially in its expanded footplate (Price, 1935; Watson, 1951, p. 134) but its absolute size is not very great, and its shaft is of the same order of magnitude as that of the bullfrog. The pelycosaur and captorhinomorph type of stapes is also characterized by the relative firmness of its attachments: the footplate often bears a distinct rim that articulates with the rim of fenestra ovalis; the dorsal process is large, well-ossified, and articulates with a depression on the paroccipital process; a presumably cartilaginous extension of the shaft is received in a hollow on the posterior face of the quadrate, the stapedial recess. There is some evidence for a distal extension (tympanic process) connected with a tympanum in a few forms (Parrington, 1946, 1955), but the existence of a tympanic process generally among the pelycosaurs and captorhinomorphs is a matter of dispute.

The postero-lateral margin of the skull of captorhinomorphs and pelycosaurs is not excavated as it is in lizards, the stapedial recess being restricted to the posterior face of the qaudrate. In *Dimetrodon* and *Ophiacodon* the retroarticular process of the lower jaw is poorly developed. Thus it is difficult to envision a superficial tympanum behind the quadrate and above the jaw joint, in which the stapes could terminate after the fashion of modern lizards. In sum, the middle ear region of most primitive reptiles does not show the highly refined adaptation to hearing seen in lizards, crocodilians, turtles, and birds.

The amphibian ancestor of the pelycosaurs presumably had a typically labyrinthodont otic notch in the dorso-posterior margin of the skull, into which the distal end of the stapes was directed (fig. 6, A). This notch is reasonably interpreted as having housed a tympanum, the

whole apparatus functioning in the reception and transmission of air-borne sound. In view of evidence that later mammal-like reptiles (descended from pelycosaurs closely related to *Dimetrodon*) also had a middle ear that detected air-borne sound by means of a tympanum (Gregory, 1910; Parrington, 1946; Watson, 1953), economy of reasoning dictates a similar interpretation of the pelycosaur middle ear. Representing a conservative viewpoint, Romer and Price (1940), Westoll (1943), and Parrington (1955) attribute to the pelycosaurs a deeply-placed ear-drum ly-





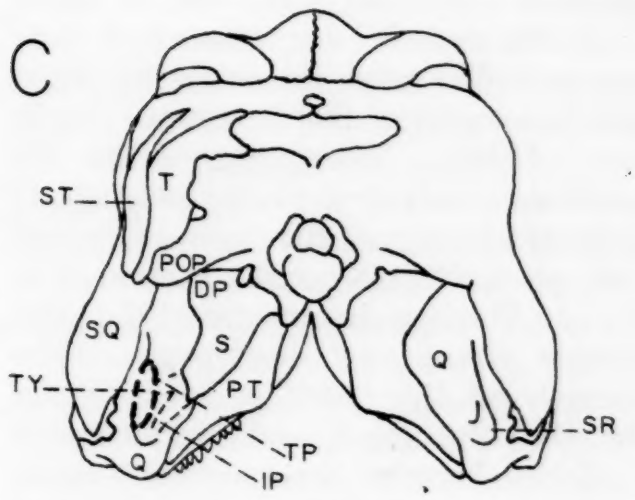


FIG. 1. Occipital views of skulls of A, lizard, *Crotaphytus collaris;* and C, pelycosaur, *Dimetrodon* sp., showing stapes, tympanum, and bones surrounding middle ear. C modified from Romer and Price (1940); tympanum placed after Parrington (1955). B, schematic diagram of model of middle ear used in experimental part of this study. Scale: A, × 2.8, B and C, × ca. 0.33. Anatomical parts corresponding to parts of model are included parenthetically with designation of model parts. Reference letters: C(DP), clamp (dorsal process); C(IP), clamp (internal process); CR, crystal cartridge (leads to oscilloscope not shown); DP, dorsal process; F(Q), frame (quadrate and squamosal); H(TP), head of rib (tympanic process); IP, internal process; L(IP), capsule of costo-vertebral joint (internal process); M(TY), cellophane membrane (tympanum); POP, paroccipital process; PT, pterygoid; Q, quadrate; RAP, retroarticular process; R(S), veal rib (stapes); S, stapes; SQ, squamosal; SP, sound source; SR stapedial recess; ST, supratemporal; T, tabular; TP, tympanic process; TY, tympanum; V(IP), fragment of transverse process of vertebra (internal process).

ing near the stapedial recess at right angles to the long axis of the stapes. Sound reaches it via an external auditory canal lying behind the squamosal and quadrate bones and in front of depressor mandibulae muscle. In this position it resembles the ear drum of lizards in its relation to quadrate, stapes and jaw joint; the chief dissimilarity is in the depth at which the drum lies in the pelycosaurs.

On the other hand, the great difference between the bony parts of the middle ear in modern reptiles and in the pelycosaurs has led Watson (1948, 1951, 1953, 1954) to conclude that the pelycosaurs, and probably also the captorhinomorph cotylosaurs, had lost the tympanum, although they retained a tympanic cavity surrounding the stapes. He does not infer that these reptiles were totally insensitive to air-borne sound, but believes that they may have been able to detect a certain amount through the broad, subcutaneously-placed bones of the cheek, with which the stapes had firm contact via the quadrate. He suggests that if sound were detected in this manner, it was transmitted through the stapes by "compression waves of infinitely small amplitude" (1954). Watson agrees that the amphibian ancestors of the pelycosaurs possessed an air-sensitive tympanum, and claims that the presence of a tympanum in the late Permian descendants of the pelycosaurs was a new development. The anomaly of this point of view lies in the close functional and morphological similarity between the tympanum-stapes mechanism of the late mammal-like reptile and that of the ancestral amphibian. If selective pressures were strong enough to force redevelopment of a tympanum-stapes complex in descendants of the pelycosaurs, one might expect them to have prevented its loss in the first place.

Tumarkin (1955) believes that Watson's interpretation of the pelycosaur middle ear implies that pre-pelycosaur amphibians also lacked a tympanum. He argues that the amphibian mode of hearing was similar to that suggested by Watson for the pelycosaurs, and that the pelycosaurs heard either in this fashion or through the buccal cavity. From a strictly logical point of view his discussion is quite convincing, but is consistently irreconcilable with paleontological evidence. For detailed criticism of this aspect of Tumarkin's earlier work (1948, 1949) see Parrington (1949) and Vaughn (1955); their comments are generally applicable to his 1955 paper. Be that as it may, Tumarkin's view of the early evolution of the middle ear is largely dependent upon the thesis that the pelycosaurs lacked a tympanum.

Watson's argument against a tympanum in the pelycosaurs follows three lines. He asserts that the shape of the quadrate precludes support of a tympanum anywhere near the end of the stapes, maintaining that the end of the pelycosaur stapes was continued in cartilage into the stapedial recess, with the former tympanic process still directed toward a drum that no longer existed. He implies that no membrane of reasonable size could move such a massive stapes, and finally, he states (Watson, 1953, p. 163) that ". . . it is very difficult to believe that a stapes so powerfully connected . . . [as that of *Dimetrodon*] could have been actuated by a tympanic membrane."

The argument that firmness of attachment would prevent the bone from being actuated by a membrane may be confuted immediately. It ignores the extremely small amplitude through which the stapes vibrates. In the human, at frequencies to which the ear is most sensitive, the amplitude of the footplate is on the order of 10^{-10} cm (Békésy and Rosenblith, in Stevens, 1951). This is the same order of magnitude as the diameter of a hydrogen molecule; even if the stapes were fused or sutured to the skull, the possibility would remain that sound could have been transmitted through the bone molecularly, by bone conduction. However, the pelycosaur stapes was not fused or sutured to the skull, as shown by the number of specimens in which the bone

is displaced or missing, so it is not necessary to invoke bone conduction because of stapedial attachments. The stapes must certainly have been free enough to permit the amplitude of movement observed in the mammalian stapes.

The central, unresolved point of much of the disagreement over whether or not the pelycosaurs had a tympanum depends upon whether or not a membrane of moderate size can actuate a large stapes. Watson implies that the pelycosaur stapes is too large to be actuated by a membrane of any reasonable size or position, and the conservative point of view, by its mere existence, implies that a membrane large enough to actuate the stapes could have been supported on the pelycosaur skull. Neither side of this aspect of the argument is backed by concrete evidence, and information is needed concerning the minimal size of tympanum capable of setting in motion a bone of the mass of, for example, the stapes of *Dimetrodon*. The following experiments were designed for the express purpose of meeting this need.

Materials and Methods

A moderately taut membrane, whatever its size, will vibrate as air-borne sound strikes it. The question is, how large a thin membrane, of relatively small mass, will be required to set up detectible vibration in a rod of bone of relatively large mass? If vibration is detected in the bone, is it transmitted at a molecular level, or does the bone vibrate bodily? The possibility that the membrane need not be of inordinately large size may be demonstrated by listening at one end of a broomstick while another person taps the other end with a short length of cotton string. A blow which cannot be heard through air over the length of the broomstick is clearly audible at the opposite end of the stick. The relatively small amount of energy that can be delivered by the string has set up detectible molecular vibration in the massive broomstick.

Figure 1, B shows the components of a series of models which were constructed to provide a rough functional approximation of the pelycosaur middle ear. The stapes was represented by two sizes of veal rib, and the tympanum by a cellophane sheet. The quadrate and squamosal bones, which must have supported the tympanum in the pelycosaurs, were represented in each model by a frame made of ⅛ in. (3 mm) plywood. Four sizes of tympanum were simulated, the diameter being determined by the size of the hole in the plywood across which the cellophane was supported; the membranes were attached to their frames by rubber cement. The wood frame was clamped to the same ringstand that supported the bone, the shaft of which was wrapped with friction tape to provide a flap that could be gripped by the ringstand clamp. The tuberculum of the rib retained a small piece of vertebra attached to it by fibrous connective tissue, which was utilized to clamp the bone to the wooden frame in such a way that the head of the rib butted firmly against the membrane.

The pressure contact between bone and membrane was a poor approximation to the living cartilaginous connection, but probably represented a closer approach to reality than a cemented connection. A cemented connection would transmit sound better than the living cartilage; any similar advantage the pressure contact might have enjoyed was probably nullified by the considerable tension of the membrane that it required. Connection of the bone to the ringstand by the friction tape represented the dorsal process, connection to the frame represented the internal (quadrate) process, and contact with the membrane represented the tympanic process (hypothetical in the pelycosaurs). With regard to these connections, the bone was probably held with about the same rigidity in the models as was the stapes in the living pelycosaur. The opposite end of the bone, left free in this experiment, represented the proximal end of the stapes. This end appears to have

been rather firmly attached to the margin of fenestra ovalis in *Dimetrodon,* and perhaps a little less so in *Ophiacodon.* It was left free for the same reason that no attempt was made to enclose the tympanic cavity; that is, since the experiment could not be more than a rough approximation, it was felt that the effort expended in elaborating the setup would not be repaid in increased accuracy or in closer resemblance to reality.

Vibration of the bone was detected by an inexpensive crystal phonograph cartridge, the top limit of sensitivity of which was about 5000 cycles per second. It was not thought necessary to test the apparatus above this level because of the low range of highest sensitivity to air-borne sound in modern reptiles. Wever and Vernon (1956), for example, have shown that the greatest sensitivity in turtles is in the range of 100 to 700 cps.

Two different techniques were used to maintain contact between the crystal detector and the bone. The first (fig. 1, B) was intended to demonstrate a molecular mode of sound transmission. The cartridge was bound with friction tape to the end of the bone but separated from it by a disc of cork, while the needle of the crystal was seated firmly on the bone. The rationale of this arrangement is that the crystal itself would move with any bodily movement of the bone, and gross differential movement between bone and crystal would be held to a minimum. Molecular motion is more readily transmitted through the needle than through the cork, and would thus be more likely to actuate the crystal than would gross movement. However, although the crystal was attached tightly enough that it did not wiggle when checked by gross manipulation, it was not immovably fixed, and the possibility remains that it may have responded to bodily movements of the bone because of its own inertia and thereby have recorded more than just molecular vibration. The second technique (not figured) was intended to demonstrate bodily movement of the bone in response to vibration of the membrane. The cartridge was mounted on a separate ring-stand, with only its needle in contact with the bone. Although maintenance of contact between needle and bone proved more difficult in this setup, and its performance proved correspondingly more erratic, the body of data is as consistent as that obtained with the first technique.

The source of air-borne sound consisted of an audio-frequency generator which drove a public-address type of amplifier and ten-inch speaker; the speaker was placed about twelve inches from the membrane being tested. Sound intensity was held constant throughout the experiment. No attempt was made to obtain an objective estimate of it, but subjectively it was not great enough to cause discomfort at any frequency to human ears within a foot of the speaker. Sound waves were delivered to the membrane, which in turn transmitted them to the bone. Impulses generated in the crystal by the vibrating bone, as described above, were delivered to an oscilloscope, from the screen of which amplitudes were estimated directly. Frequencies read from the oscilloscope screen corresponded closely to settings on the audio-generator during all runs in the experiment.

The amount of vibration reaching the substrate from the speaker was recorded by placing the needle of the crystal directly on the table-top and then generating the range of frequencies used. By this means it was found that although substratal vibration of low frequency showed high amplitude, the amplitude dropped abruptly between 400 and 500 cps, and was negligible above that range. Vibration reaching the substrate was greatly reduced by placing a sponge rubber pad beneath the speaker. Thus, although some of the vibration below 500 cps may have reached the apparatus via the substrate, most of it was air-borne. Nearly all of the sound recorded from the models above that level was air-borne.

Table 1 gives dimensions of the bones and membranes used in the experiment.

TABLE 1. *Dimensions of components used in sound experiments, and ratios (bone volume/membrane area) of combinations*

Designation	Bone dimensions	Bone volume	Membrane diameter	Membrane area	Ratio
Bone (letter)					
L	120 × 20 × 10 mm	24,000 mm^3			
S	75 × 20 × 10 mm	15,000 mm^3			
Membrane (number)					
1			19 mm	284 mm^2	
2			37 mm	1034 mm^2	
3			48 mm	1809 mm^2	
4			64 mm	3217 mm^2	
Bone-membrane combination					
L1					85 : 1
L2					23 : 1
L3					13 : 1
L4					8 : 1
S1					53 : 1
S2					14 : 1
S3					8 : 1
S4					5 : 1

The smaller bone is fairly close to the proper dimensions of a very large *Ophiacodon* stapes described by Romer and Price (1940), although it is probably two or three times as massive as the reptile "ossicle." The larger bone was used in the present experiment merely to afford a basis for comparison and is not intended to represent the size of stapes of any fossil reptile; it is up to four times more massive than the *Ophiacodon* stapes. The ratio bone volume/membrane area provides a convenient index to the size relation between parts of the middle ear; the ratio is useful from a functional point of view because its components are related to the masses of their respective parts. Although the ratio represents an order of magnitude rather than a precise value when used for its functional implications, calculation of a ratio based upon masses would have been no closer to reality and would have involved more effort. Combinations of bone and membrane are designated in tables 1 and 2, and in figures 2 through 5 inclusive, by the letters used to indicate bones combined with the numbers used to indicate membranes. The suffix letters enclosed in parentheses, as used in table 2 and in figures 2 through 5 refer to the mode of support of the crystal detector; suffix "x" denotes runs in which the detector was supported on the bone, and suffix "y" runs in which the detector was supported separately from the bone.

For each combination of membrane and bone tested, the same setup of wood frame and bone was tested without the membrane in place, as a control. In the graphs shown in figures 2, 3, and 4, the white portion of the curve represents airborne sound picked up by the membrane and frame and transmitted through the bone to the crystal. The curves are composite, made up of several runs of the same combination of bone and membrane. The apparatus was torn down each time and set up afresh for each run, and although there was considerable variation in overall amplitude from one run to an-

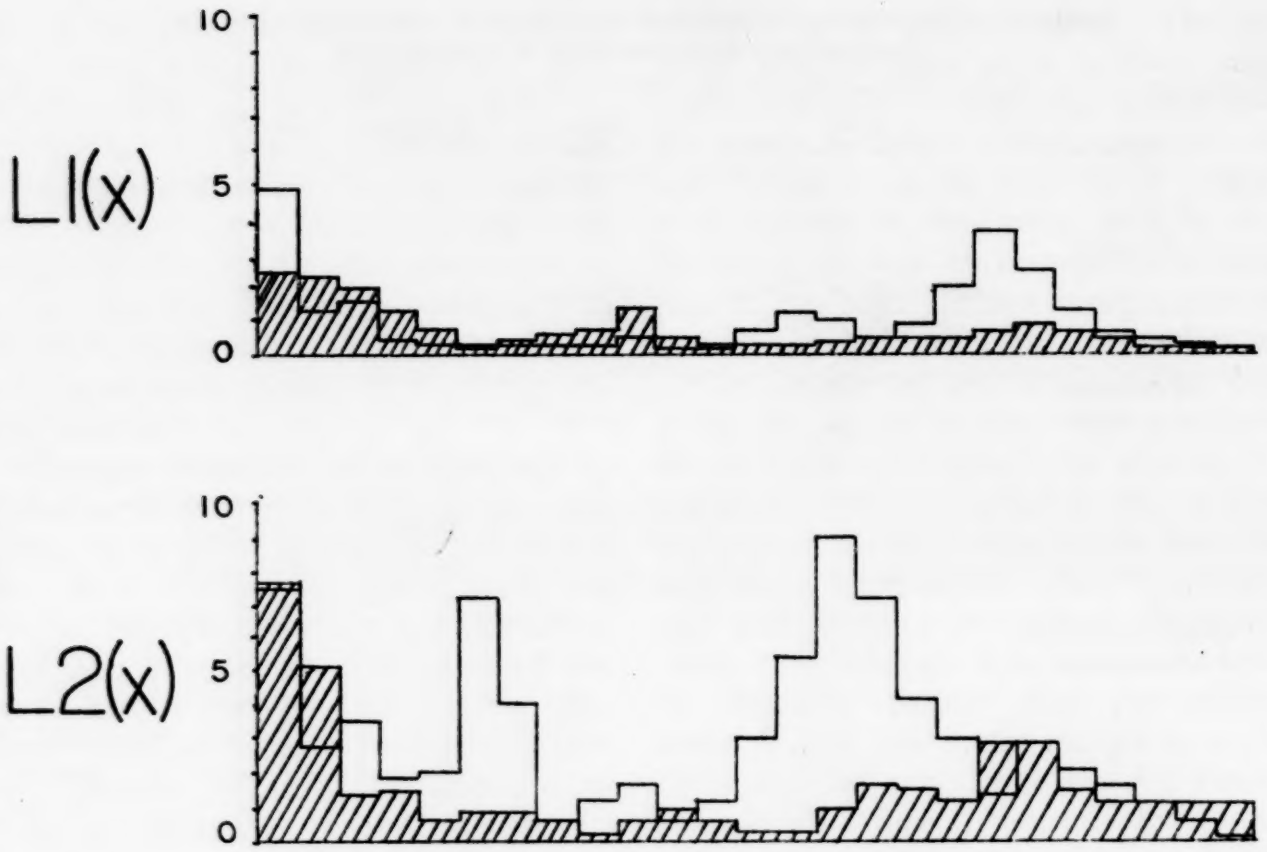


FIG. 2. Vibration detected in bone with membrane in place (clear area) compared with that detected without membrane (cross-hatched area). Abscissa shows frequency in cps × 100; ordinate shows amplitude in milivolts. Series L(x); crystal cartridge mounted directly on large bone.

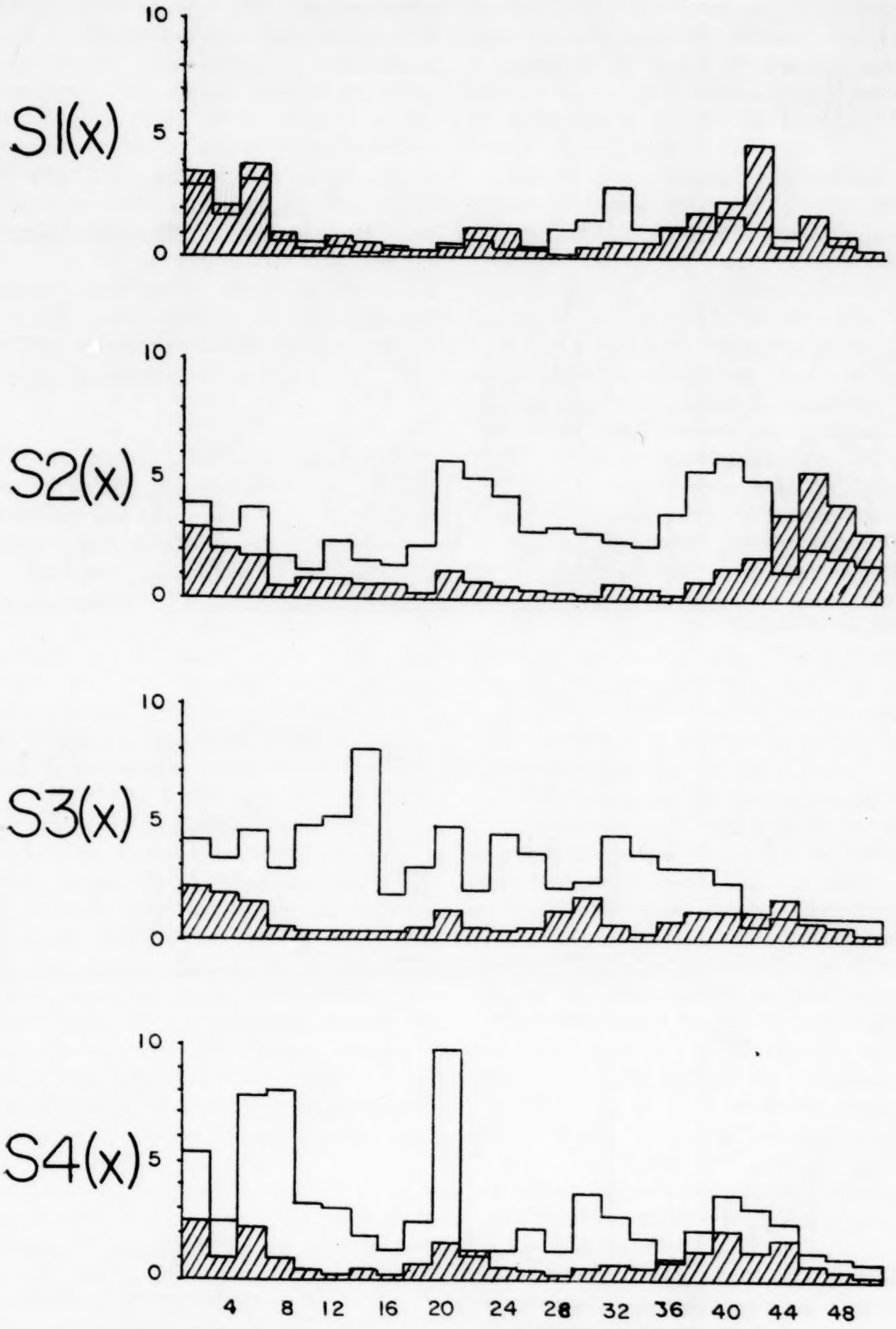


FIG. 3. Information as in figure 2. Series S(x); crystal cartridge mounted directly on small bone.

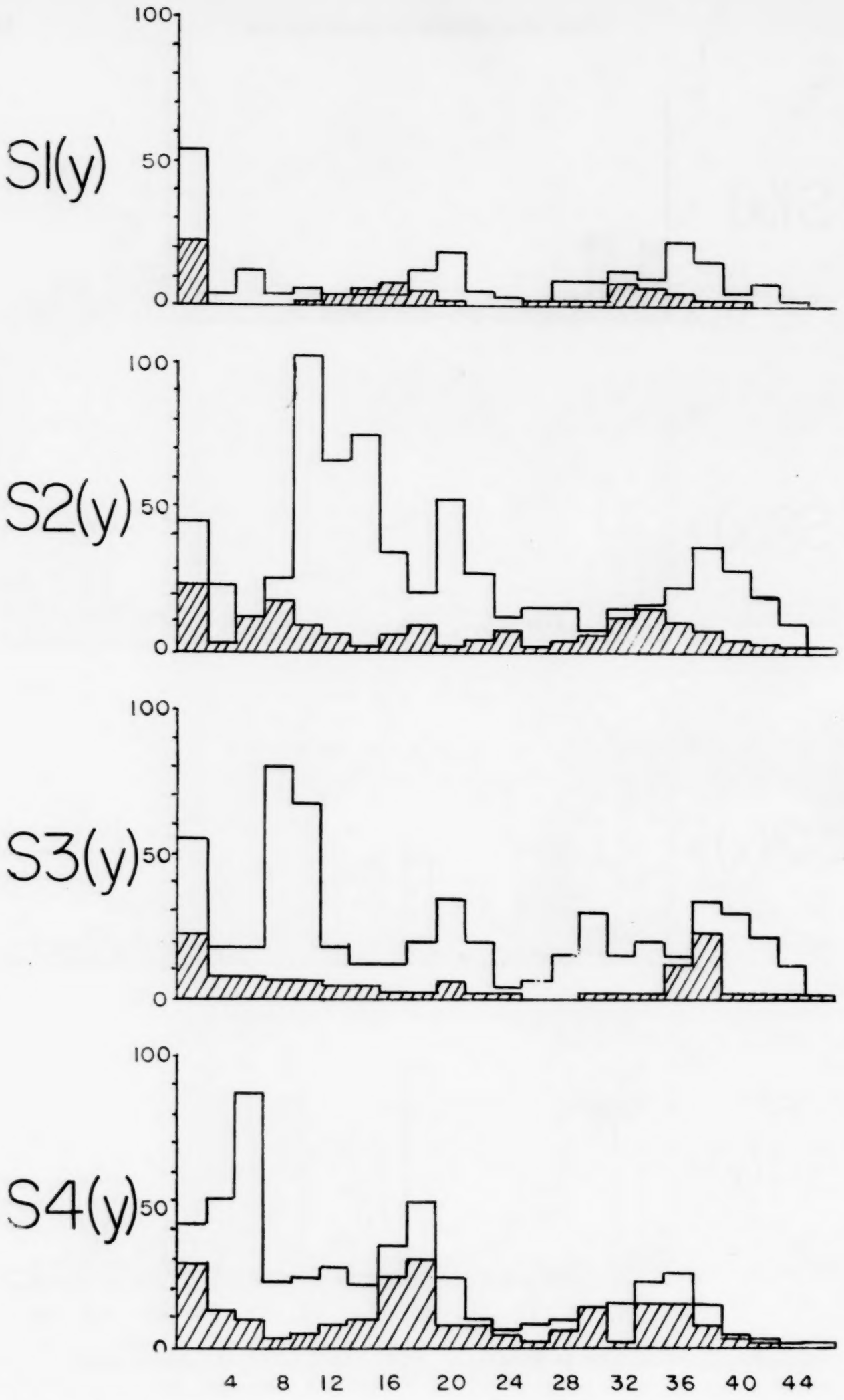


FIG. 4. Information as in figures 2 and 3. Series S(y); crystal cartridge mounted separately from small bone.

other, points of high and low amplitude were consistent between runs of the same combination. The complex patterns of resonance that appear are to be interpreted as a result of the crudity of the apparatus, and not as any intrinsic feature of a middle ear with a large stapes. There is some tendency for the larger membranes to show their greatest resonance at lower frequencies than do the smaller membranes, which indicates that the resonance patterns are characteristic of the models themselves, and not of the electronic apparatus.

Results

A comparison between amplitudes of the white and cross-hatched areas of each combination (figs. 2–4) shows that in general, the apparatus was more sensitive to air-borne sound with the membrane in place than without it, throughout most of the frequency range. Number 1, the smallest in the series, is the only membrane that was too small to actuate the larger bone (L) to an appreciable degree, or to initiate significant molecular vibration in the smaller bone.

It should be noted that the amplitudes generated by combinations of the S(y) series (fig. 4) are from seven to ten times those generated by the S(x) series (fig. 3) and the L(x) series (fig. 2). This is probably due to the detector being free of the bone in the "y" series, and thus better able to detect bodily movement than when it was mounted directly on the bone as in the "x" series. It provides strong evidence that the membranes under investigation were quite capable of causing at least the smaller of the two bones to move bodily.

When the detector was mounted directly on the bone, there was no general increase in amplitude of the combinations using the smaller bone (fig. 3) over those using the larger (fig. 2). In theory, a purely molecular mode of transmission of sound is dependent upon density of the bone and the distance the sound must travel in the bone. Density is the same in the large and small bones, and the difference in distance travelled is negligible, so that if the mode of transmission is purely molecular, no difference in performance should be expected between bones L and S.

On the other hand, transmission of sound by bodily movement of the bone should be more effective in a smaller bone, which because of its smaller mass is more readily moved. The possibility that some bodily movement was recorded in the "x" series as a result of the inertia of the detector must be recognized; that a certain amount did in fact occur is suggested by the better performance of combination S2(x) relative to combination L2(x) (fig. 5). A factor that militates against a clearer demonstration of the superiority of the small bone for sound transmission by bodily movement, in the arrangement of series "x," is that when the detector is fixed firmly to the bone its mass is added to that of the bone. The difference between masses of bones L and S is thus materially reduced, and any bodily movement recorded is not outstandingly different from one bone to the other.

Performance of the various combinations of bones and membranes is summarized in table 2, in terms of average amplitude with membrane in place; of average amplitude without membrane; and of the product of the former value divided by the latter, intended to indicate degree of improvement of performance with membrane relative to performance without membrane (differential). The differential is interpreted as a measure of the efficiency of the membrane itself in actuating the bone to a detectible degree. Figure 5 demonstrates regions of the frequency range in which each combination functions most effectively, evaluated in terms of membrane efficiency.

Table 2 shows that each size of membrane except the smallest increases slightly in efficiency when used in conjunction with the smaller bone, when the detector is mounted directly on the bone (series "x"), even though the average

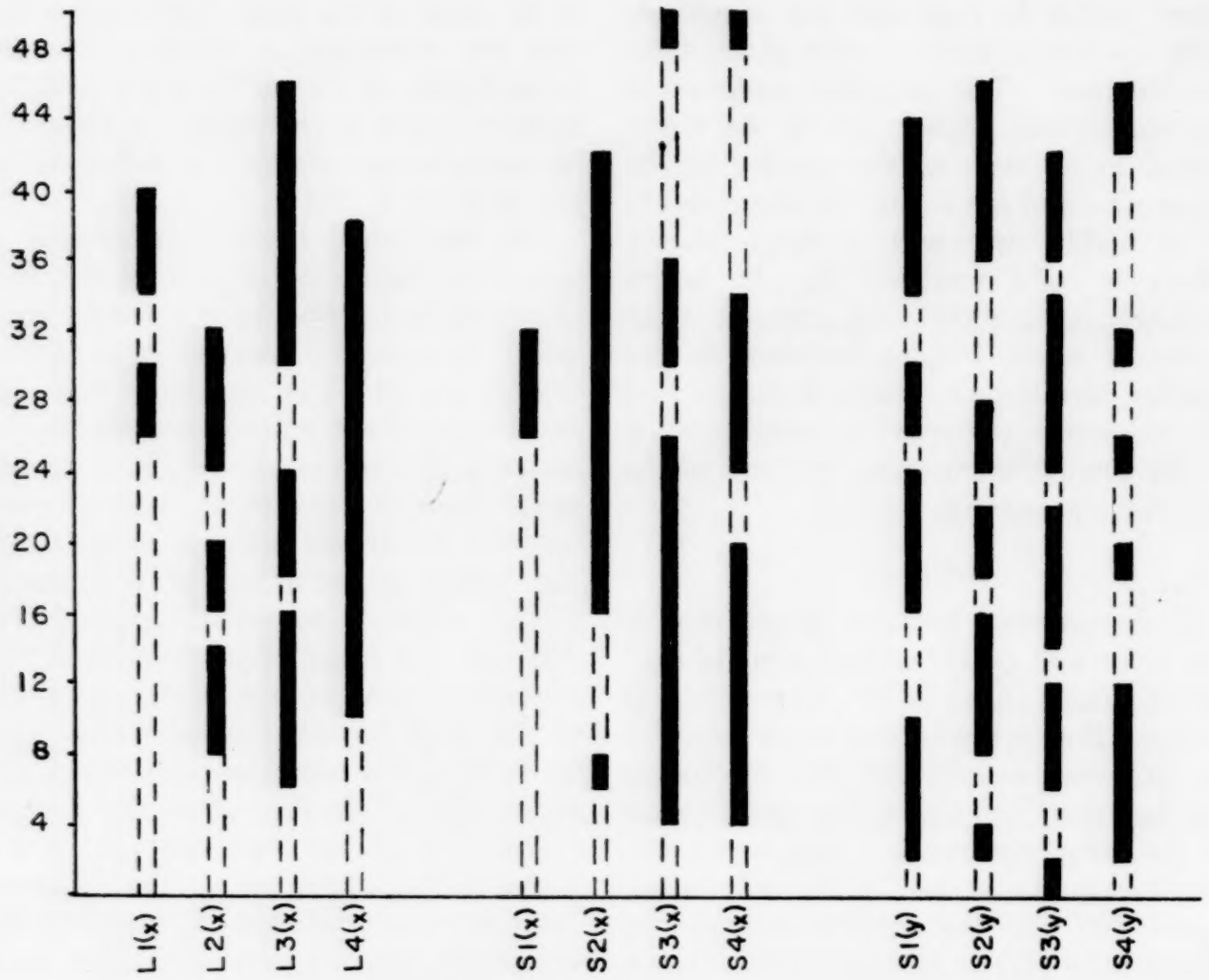


FIG. 5. Frequencies at which amplitude detected with membrane is at least three times that detected without membrane. Ordinate shows frequencies in cps × 100.

TABLE 2. *Average amplitude of bone-membrane combinations, in millivolts, as read from oscilloscope trace*

Combination	With membrane (I)	Without membrane (II)	Differential $\left(\frac{I}{II}\right)$
L1(x)	1.0	.6	1.7
L2(x)	3.2	1.6	2.0
L3(x)	3.7	1.3	2.8
L4(x)	3.8	2.0	1.9
S1(x)	1.1	1.3	.8
S2(x)	3.1	1.4	2.2
S3(x)	3.1	.8	3.9
S4(x)	2.9	.8	3.6
S1(y)	9.2	2.4	3.8
S2(y)	29.7	7.2	4.1
S3(y)	24.1	4.4	5.5
S4(y)	22.3	8.8	2.5

amplitude is slightly smaller when the small bone is used. Efficiency of all sizes of membrane is greatly increased at the higher amplitudes recorded when the bone is allowed to move completely independently of the detector (series "y").

In the arrangement of series "x," membrane number 3 turns in generally the best performance, being most efficient with either size of bone, and producing a high average amplitude. Membrane number 1 is probably ineffective in series "x." In transition from the larger to the smaller bone, membrane number 2 improves slightly and number 4 greatly, in efficiency, although the average amplitude of number 2 rises while that of number 4 drops. The performance of membranes 2 and 4 in series "x" are approximately comparable, but it may be significant that

the smaller membrane (and frame) records a higher amplitude with the smaller bone than with the larger.

In series "y," membrane number 3 is still the most efficient, but the improvement in average amplitude as well as efficiency of number 2 indicates that it should rank with number 3 in terms of overall effectiveness. Membrane number 1 shows the greatest improvement in efficiency in transition from series "x" to series "y," and considerable improvement in average amplitude, but remains the least effective of the four membranes tested.

A striking feature of membrane number 4 is its drop in efficiency in transition from series "x" to series "y." Its average amplitude has risen in the transition, but not as much as that of number 2 and no more than that of number 3. If efficiency were solely a matter of size of membrane relative to size of bone, one would expect number 4 to turn in consistently the best performance of the four, but it has not done so. A possible explanation of this failure is that number 4 is too large for the low level of sound intensity with which the membranes were tested. The smaller membranes failed to perform well with the largest bone because they could not gather enough energy from the sound presented to them to produce high amplitudes of vibration in the bone. On the other hand, although more energy would impinge against its broader area, membrane number 4 would require more energy merely to set it in motion and might have less energy, proportionate to what it received, available to actuate the bone. Because of this energy loss, at a low level of sound intensity number 4 might be working at close to its maximum effectiveness with the larger bone and less efficient detector arrangement. While the effectiveness of the other membranes would improve in combination with the smaller bone and more efficient detector arrangement, number 4 would still be faced with its energy loss and consequently its combinations would fail to improve to the same degree as those of the other membranes. If a higher level of sound intensity had been used, undoubtedly number 4 would have proved best in both average amplitude and in membrane efficiency, in all combinations.

Another series of experiments was run paralleling those described above, using bones and membranes of the same sizes, but having the membranes supported only part way around the periphery, in an attempt to simulate the type of membrane support found in the lizard. Results were roughly parallel to those of the experiments described in detail, but were not as reliable because of a smaller number of runs. The only safe conclusions to be drawn from this series are that the apparatus is more sensitive to air-borne sound with membrane than without, and that a partially supported membrane is not as effective as a completely supported membrane of the same size.

Conclusions Based on Experimental Results

When all features shown in table 2 are taken into consideration, the most effective bone-membrane combinations are seen to be S3(x and y), S2(y), and L3(x). Table 1 shows that the bone/membrane ratios of these combinations lie between 14:1 and 8:1. The greatest improvements in the course of the experiment are those of the transition from L2(x) to S2(y), in which the bone-membrane ratio changes from 23:1 to 14:1, and the transition from L1(x) to S1(y), in which the ratio changes from 85:1 to 53:1. If the performance of membrane number 4 be ignored for reasons set forth above, it appears that the maximum effective bone/membrane ratios for a stapes from three to five times the mass of that of a large pelycosaur lies somewhere below 23:1 and above 14:1. The improvement of performance in transition from L1(x) to S1(y) is not highly significant because of the relatively low average amplitude of S1(y), but it does suggest that the upper limit of the effective

TABLE 3. *Possible membrane diameters in pelycosaurs*

Bone/membrane ratio	Ophiacodon	Dimetrodon
8 : 1	19 mm	30 mm
14 : 1	14 mm	22 mm
20 : 1	12 mm	19 mm

ratio may be poorly defined, particularly when the stapes is smaller than the bones used in the experiment.

The suggested tympanum diameters listed in table 3, based upon experimentally-derived ratios, are of proper order of magnitude to lie behind the quadrate, at the end of the stapes, and in a plane at right angles to the stapes ("normal" reptilian position) in the pelycosaurs. Dimensions used to determine stapes volume for use in table 3 were derived from casts of *Ophiacodon uniformis* (MCZ 1366) and *Dimetrodon limbatus* (MCZ 1347). Figure 1, C, based upon a reconstruction by Parrington (1955, fig. 14 B), shows the skull of *Dimetrodon* in occipital aspect with a tympanum in this position; ratio of volume of the bony part of the stapes to area of tympanum in this diagram is between 15:1 and 20:1. Westoll (1943, fig. 5 C), in a similar diagram, shows a tympanum to whose area the volume of the stapes bears a ratio of from 5:1 to 7:1. It is very probable that these tympana, postulated on morphological grounds by Parrington and by Westoll, are entirely large enough to have been able to actuate the stapes with which they are associated.

Parrington (1955, p. 33) estimates the tympanum of *Dixeya,* a small gorgonopsian, to have had an area of "at least 18 to 20 sq. mm," and in figure 8 of the same paper he shows the complete stapes of *Scylacops,* another gorgonopsian of about the same size as *Dixeya.* The ratio of volume of *Scylacops* stapes to area of *Dixeya* membrane is from 15:1 to 17:1. Watson (1951, p. 163) estimates the tympanum area of a small gorgonopsian at from 10 to 15 sq. mm; this animal is about the same size as *Scylacops* and *Dixeya,* and appears to have had a stapes similar in most respects, including size, to that of the former. The bone/membrane ratio in Watson's animal must range between 20:1 and 30:1. The maximum effective ratio for bones the size of those used in the experiment is on the order of 23:1, but as suggested above, the upper limit of the ratio is not well-defined when smaller bones are employed. It is quite probable that with the smaller size of the gorgonopsian stapes, the effective ratio is appreciably higher than in the pelycosaurs, and that even the high ratio occasioned by Watson's more conservative estimate of membrane area is within the limit for effective reception and conduction of air-borne sound.

POSITION OF THE TYMPANUM IN THE LARGE PELYCOSAURS

The experimental models were constructed as though the pelycosaur tympanum lay in a typically reptilian position with respect to the stapes, i.e., terminal to it and in a plane roughly at right angles to its long axis. This assumption is determined largely by analogy with the relationship of tympanum to stapes in living reptiles, and, in large pelycosaurs like *Dimetrodon* and *Ophiacodon,* morphological evidence favoring it is rather scanty. The distal end of the stapes is featureless, and is usually considered to have been continued in cartilage. Watson (1955) asserts that this cartilage extension was directed toward a pit in the bottom of the stapedial recess, where it ended; he speaks of the tympanic process as being directed toward a tympanum that no longer exists. Romer and Price (1940) and Parrington (1955) disagree with this point of view. The former content that the cartilage extension was not directed into the stapedial recess but passed laterally just above its deepest part as a tympanic process, to make contact with a tympanum attached (in part) near the lower margin of the stapedial recess. Romer believes that an elongate hollow

on the lower surface of the bony stapes of *Dimetrodon* marks the attachment of a ligament that ran to the depths of the stapedial recess as an internal process. Parrington agrees generally with Romer on the direction of the cartilage extension and the position of the membrane but diagrams the cartilaginous part of the stapes as though it were bifurcate, one branch going to the tympanum and the other into the stapedial recess (fig. 1, B).

Romer (*op. cit.*, p. 88) describes the bony stapes of *Dimetrodon* as follows:

> The shape of the shaft is such that the basal portion is rather thin from side to side and deep dorso-ventrally. Shaft and dorsal process are in a single plane; the footplate is turned medially about 45 degrees. In the articulated skull the shaft of the bone runs backward and outward and slightly downward to terminate internal to the pterygoid and quadrate near the lower margin of these elements.
>
> The dorso-lateral margin of the stapes is thinned out to a distinct crest, as though for attachment of a membrane; the crest runs up onto the laterally-directed margin of the flattened dorsal process to a level approximately even with the tip of the paroccipital process. At this point the margin of the dorsal process curves dorso-medially beneath the paroccipital process, and this part of it is not crested but rounded.

The cartilaginous extension of the stapes is not preserved, and since the opposing theories of Watson and Romer depend upon different interpretations of it, they cannot be resolved. The general shape and orientation of the bony stapes, its antero-posterior flattening, and the crest on its dorso-lateral border provide evidence in support of a third possibility for position of the tympanum in large pelycosaurs. It is here suggested that the crest on the shaft and dorsal process of the stapes formed the medial support of the tympanum in *Dimetrodon* (fig. 6, B). The dorsal margin of the tympanum curved laterally beneath the tip of the paroccipital process after leaving the dorsal process, and passed to the squamosal where it found its lateral support below the tip of the tabular. Running downward, the border of the membrane turned medially to cross the quadrate just above the stapedial recess; ventral support of the tympanum was on the quadrate and cartilaginous extension of the stapes (which probably did enter the stapedial recess as Watson believes). The stapes of *Ophiacodon* is not as flattened as is that of *Dimetrodon,* but bears a crest in the same position, so that a tympanum was probably supported in the same way in both animals. Relationships between stapes, paroccipital process, squamosal, and stapedial recess suggest that *Edaphosaurus* also had a tympanum supported in this fashion.

In modern reptiles with a tympanic cavity there is a "mesostapes," a thin membrane composed of the mucous lining of the tympanic cavity, that runs from the dorsum of the stapes up to the paroccipital process. It may be argued that the great size of the pelycosaur bone might have resulted in the mesostapes being reinforced to form a ligament that would account for the stapedial crest of *Dimetrodon* and *Ophiacodon.* However, the strength of the dorsal and internal processes should certainly have provided enough support by themselves; it is doubtful that a ligament inserting on the crest could have provided much additional support for a vertically oriented stapes like that of the large pelycosaurs.

A tympanum supported medially by the stapes and laterally by the squamosal would lie nearly at right angles to the long axis of the skull. The external auditory meatus would enter the side of the head between the squamosal and the depressor mandibulae muscle just below the tip of the tabular and paroccipital process, as suggested by Parrington (1955). It would be developed into a canal that passed medially as far as the medial edge of the stapes where it ended blindly; the tympanum then would form a part of the anterior wall of the external canal rather than its terminus. The

middle ear cavity would lie in front of the tympanum and stapes and behind the quadrate; it would blend indistinguishably with a narrow but deep Eustachian canal behind the pterygoid. The relation between tympanum and external auditory canal is rather unusual in reptiles, in which the external canal, if it exists, is oriented nearly at right angles to the plane of the membrane. In mammals, however, the canal frequently meets the tympanum at such a shallow angle that the latter may be thought of as making up part of the roof of the canal.

Function of Tympanum Supported by Stapes

A tympanum lying in the same plane as the flat stapes must have vibrated at right angles to the plane and long axis of the bone. The stapes, although it provided about half of the support for the tympanum, was free to move slightly, while the squamosal was fixed, so that any vibration set up in the membrane was transmitted directly to the stapes. The proximal end of the dorsal process of a *Dimetrodon* stapes at hand (cast, MCZ 1347) has a smooth condylar surface, and looks as though its articulation with the paroccipital process were diarthrodial in nature. The condyle is elongated in the plane of the dorsal process (and of the stapes); it is clearly adapted to permit movement at right angles to the plane of the bone. Vibration of the membrane caused the stapes to vibrate antero-posteriorly, pivoting on the paroccipital articulation and producing a rocking motion at the footplate like that described in mammals. This type of movement must have utilized a lever at least as long as the depth of the dorsal process, and conceivably nearly the full length of the shaft of the stapes. A rocking motion in a footplate of the size of *Dimetrodon*'s need not have been of very high amplitude to have produced a disturbance in the perilymph; indeed, in the light of evidence of bodily movement of the large pelycosaur stapes, it almost appears that the inner ear apparatus must have been of extraordinary coarseness.

Evolutionary Implications

The middle ear of the labyrinthodont amphibians, among which are numbered the ancestors of pelycosaur and captorhinomorph reptiles, appears to have been of much the same nature as that of modern reptiles, a lateral outpouching of the pharynx. It lies high on the back of the skull; its bony boundaries are pterygoid and squamosal anteriorly, and paroccipital process and tabular dorsally. In life it was bounded posteriorly by a membranous sheet backed by muscle, and laterally by the tympanum stretched across the otic notch. The otic notch consists typically of a gap in the postero-dorsal margin of the skull, bounded above by the supratemporal and tabular bones of the skull table, and below by the squamosal of the cheek region. The paroccipital process is short, and slants upward to gain contact with a prominent ventro-medially directed process of the tabular. The quadrate lies below the level of the otic capsule, sheathed on its lateral side by the squamosal and guadratojugal bones of the cheek, and on its medial side by the pterygoid. The amphibian stapes extends across the tympanic cavity from the lateral wall of the otic capsule to the tympanum. A bony dorsal process passes up to the tip of the paroccipital process, and a ligamentous internal process passed in life downward to the quadrate; the remainder of the shaft, which attaches to the tympanum, is designated tympanic process. Figure 6, A represents the skull of *Paleogyrinus,* a primitive anthracosaur amphibian, in occipital aspect. Tympanum and stapes have been placed in the diagram solely on the basis of bony landmarks. Watson (1953), in a similar restoration of the stapes of *Rhinesuchus,* a temnospondylous amphibian, shows the internal process coming off the shaft more medially, so that the tympanic process is considerably longer than that of *Paleogyrinus.* The actual length of the tympanic process is

not significant of itself; the important feature is that the amphibian quadrate lies below the level of the otic capsule, so that the internal process must slant downward no matter where it comes off the stapes. *Paleogyrinus* was selected to represent the amphibian condition of the stapes because as an anthracosaur it lies closer than any

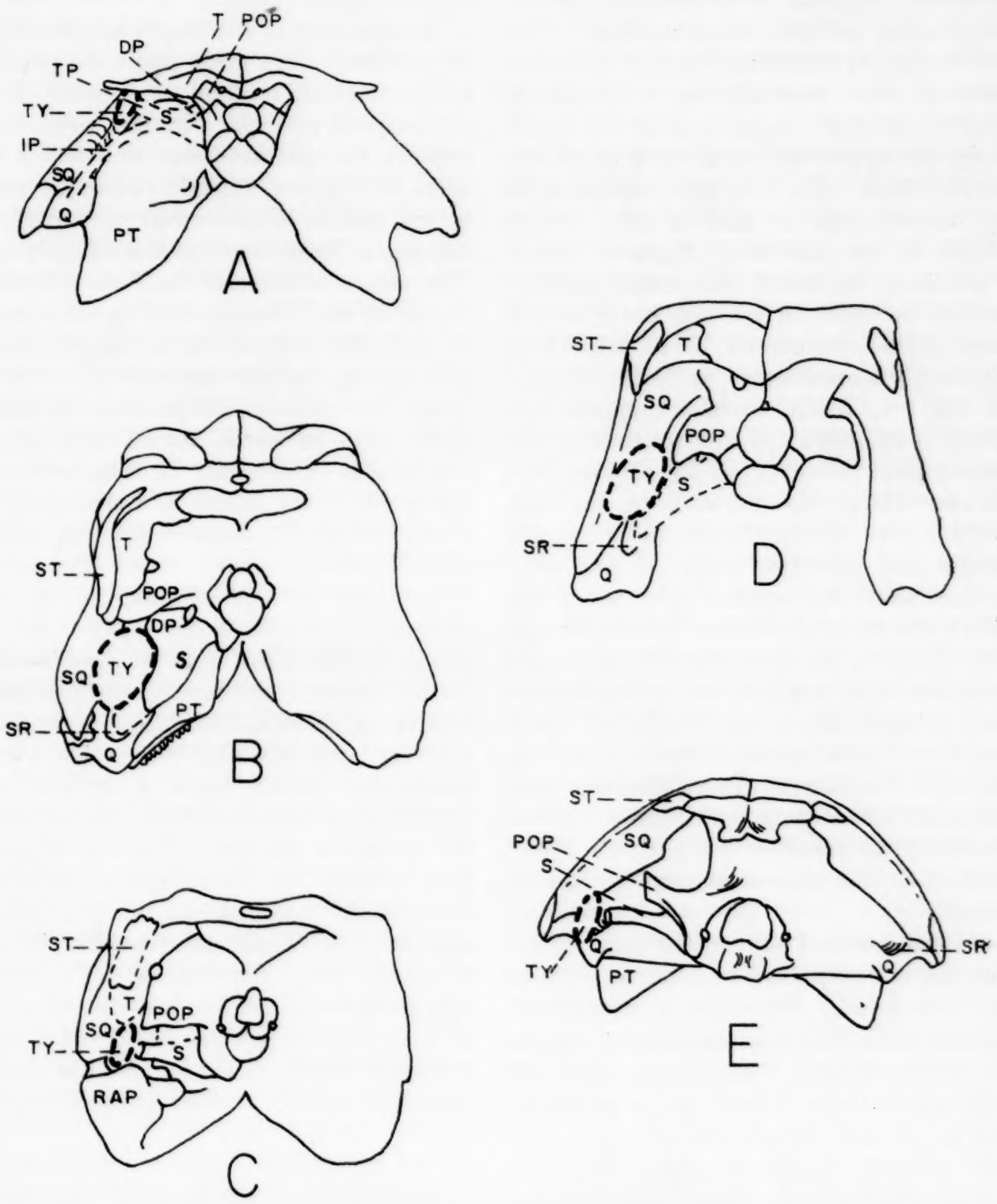


FIG. 6. Series of skulls in occipital aspect, illustrating possible changes in attachment of tympanum during closure of otic notch and evolution of pelycosaur and captorhinomorph middle ear. Not to scale. A, *Paleogyrinus,* anthracosaur amphibian; B, *Dimetrodon,* large (spenacodont) pelycosaur; C, *Varanops,* small (sphenacodont) pelycosaur; D, *Protorothyris,* primitive captorhinomorph; E, *Captorhinus,* advanced captorhinomorph. A from Watson (1926), B and C from Romer and Price (1940), D from Watson (1954); all modified slightly. E, restoration of specimen number KUMNH 9978. For reference letters see figure 1.

temnospondyle to the stem from which all reptiles sprang.

In transition from an amphibian to a reptilian condition of the skull, the quadrate bone came to stand vertically, possibly in response to a need for firmer buttressing of the suspensorium. The definitive pelycosaur and captorhinomorph pattern was attained by a backward movement of the upper end of the quadrate; the upper border of squamosal was carried back with it to gain contact with the supratemporal and tabular bones, which at the same time migrated downward and backward, the entire process serving to close off the otic notch and to deprive the tympanum of its superficial support. Concomitant with the closure of the otic notch, certain changes took place in relationships and attitude of the paroccipital process. Supratemporal and tabular bones lay dorsally in the amphibian, but as they came to lie on the lateral and posterior aspect of the pelycosaur skull the ventral process of the latter was reduced, and the tabular margin that formerly bounded the otic notch and provided some support for the tympanum was brought into contact with the tip of the paroccipital process (cf. A and B, fig. 6). The latter, meanwhile, was bent downward to a contact with the top of the quadrate; in some forms, such as *Dimetrodon,* it has increased considerably in length.

The downward shift of the paroccipital process is interpreted by Watson as having "crowded" the shaft of the stapes (presumably the tympanic process) ahead of it, downward toward the quadrate, where it became lodged in the stapedial recess. A corollary of his scheme is that with closure of the otic notch the tympanum disappeared. Romer and Parrington agree with Watson that the tympanic process of the stapes moved downward ahead of the paroccipital process, but believe that the tympanum, instead of disappearing, passed downward along the quadrate with the tympanic process, coming eventually to lie near the stapedial recess. Romer and Watson agree that the internal process in the pelycosaurs was probably ligamentous, Romer directing it into the stapedial recess and Watson to the junction between quadrate and pterygoid bones.

The attitude of the stapes appears to be more nearly dependent upon the position of the quadrate than on the attitude of the paroccipital process. In the large pelycosaurs the quadrate lies well below the level of the otic capsule, while in small forms and in *Captorhinus* the quadrate lies at the same level as the otic capsule. The stapes terminates near the stapedial recess in both forms, slanting downward to reach the quadrate in the large animals and passing straight laterally in the small ones. The paroccipital process also slants downward in some forms and passes laterally in others, but its relationship to the angle of the stapes is not consistent. For example, in *Dimetrodon* the paroccipital process slants downward more steeply than in *Ophiacodon,* and yet the angle of the stapes in the latter is if anything steeper than it is in *Dimetrodon.* In its constant relation to the quadrate, and in its downward-slanting attitude in the large animals, the bony shaft of the pelycosaur stapes bears a striking resemblance to the amphibian internal process, to which the present writer suggests that it is entirely homologous. The low level of the quadrate in the generalized amphibian skull indicates that this position is primitive in pelycosaurs. The slight exaggeration of the depth of the quadrate of large pelycosaurs has preserved a more primitive aspect of the middle ear region than that seen in smaller members of the order.

The attachment of one side of the tympanum along the shaft of the stapes instead of to a specific tympanic process can be shown to follow naturally from the mode of closure of the otic notch, and to have influenced strongly the change of shape of the stapes from amphibian to pelycosaur. In the amphibian the tympanum was attached to the squamosal

along one side of the otic notch and to the supratemporal and tabular bones along the other side. The dorsal position of the latter placed the otic notch a considerable distance away from the paroccipital process and dorsal process of the stapes, so that a tympanic process was necessary. As the otic notch closed, the tabular and supratemporal were brought closer to the paroccipital process and dorsal process, carrying the tympanum with them so that the tympanic process was shortened. By the time the notch was completely closed, the originally dorsal attachment of the tympanum (to the tabular) had been transferred downward and medially to the dorsal and internal processes of the stapes. Direct attachment of part of the ear-drum to the stapes obviated any need for a tympanic process, which was thus shortened out of existence. The primitively ventral attachment of the ear-drum, to the squamosal, remained essentially as it had been in the amphibian, except that with the new orientation of the tympanum the squamosal attachment was now lateral. At the same time that the tympanic process was being reduced and the tympanic attachment was being transferred from supratemporal and tabular to stapes, it must be assumed that the formerly ligamentous internal process was becoming progressively ossified. Presumably ossification of the internal process was brought about by the need for support of one side of a good-sized tympanum; support of a large tympanum may be sufficient reason of itself to account for the large size of the pelycosaur stapes. The altered appearance of the stapes in transition from amphibian to pelycosaur is not the result of a horizontal tympanic process being forced into a vertical position. Rather it is the result of emphasis of a vertical axis through internal process and footplate that was present but undeveloped in the amphibian, accompanied by suppression of the amphibian horizontal axis through tympanic process and footplate.

According to this view, the condition of the middle ear in the small pelycosaurs and *Captorhinus* (fig. 6, C and E) is a more advanced development, brought about by reduced depth of the cheek, which raised the quadrate to the level of the otic capsule and moved the stapes into a horizontal position. In the primitive vertical position, the shaft of the stapes was parallel to the posterior margin of the squamosal, and there was room between the two bones for a tympanum, but as the shaft of the stapes moved upward it came to be pointed toward the squamosal rather than having its length parallel to it, and came to be parallel to the paroccipital process. This drastically curtailed the space available for suspension of a tympanum in the manner of *Dimetrodon.* The ear-drum must have been forced toward the tip of the stapes, so that it came to lie below the squamosal and behind the quadrate just above the jaw joint, as it does in modern reptiles. In *Captorhinus* the posterior face of the squamosal is slightly concave and arches above the distal end of the stapes. A slight crest at the ventral end of squamosal runs into the margin of the stapedial recess and may mark the attachment of a terminal tympanum. A tympanum supported by this crest would provide a bone/membrane ratio of not more than 2:1. Indirect evidence for a tympanum terminal to the stapes may be inferred from the posteriorly-projecting retroarticular process in *Varanops* and *Captorhinus,* which may have served to hold depressor mandibulae away from the tympanum. No posterior projection of the articular region is developed in the lower jaw of the large pelycosaurs, perhaps partly because the tympanum is not terminal and would not be interfered with by a depressor mandibulae lying close to the back of the squamosal. The tympanum undoubtedly retained contact with the distal end of the stapes, probably through evolution of a neomorphic cartilaginous tympanic process that sprang from the dorsal side of the shaft of the stapes, which, as the homologue of the internal

process, retained its old contact with the quadrate.

The vicissitudes suffered by the tympanum during the evolution of the tetrapod skull from an amphibian to a reptilian pattern may be summarized in terms of three morphological stages, each of which is represented by well-known fossil tetrapods. In the first stage, represented by any generalized labyrinthodont, the tympanum is superficial in position, supported in the otic notch high on the back of the skull. The second stage results from closure of the otic notch and concomitant shortening of the tympanic process of the stapes, and is represented best by large pelycosaurs like *Dimetrodon* and *Ophiacodon*. In the second stage the tympanum has been forced out of its superficial position and has come to lie deep on the posterior surface of the skull, below the paroccipital process; its tabular and supratemporal support has been exchanged for attachment along the dorsal margin of the now-flattened stapes, but its primitive amphibian attachment to the squamosal is retained. Another characteristic of the second morphological stage is the primitively low position of the quadrate relative to the otic capsule; this feature may be somewhat exaggerated in the large pelycosaurs. Transition to the third stage is the result of reduced depth in the cheek region which raises the quadrate to the level of the otic capsule, bringing the shaft of the stapes into a horizontal position. In the third stage, represented by small pelycosaurs like *Varanops,* and by *Captorhinus,* the eardrum has migrated into a terminal position with respect to the stapes. It is still deeply placed, but has come to lie behind the quadrate and above the jaw joint; its relationships are now very suggestive of the relationships of the eardrum in modern reptiles, or, in parallel fashion, in late therapsids.

The examples used to illustrate the foregoing morphological stages do not, of course, represent a phyletic line; it would be ridiculous to claim *Dimetrodon* or *Ophiacodon* as a form ancestral to *Captorhinus*. Yet, if the stages do actually mark the course followed in the evolution of the middle ear, it must be assumed that animals in which the quadrate lay well below the level of the otic capsule, requiring the stapes to slant downward, occurred in the captorhinomorph line. Until recently, captorhinomorphs more primitive than *Captorhinus* itself were poorly known, but Watson (1954) has published reconstructions of *Protorothyris* and *Romeria,* from the lowest vertebrate-bearing beds of the Texas Permian, in which the posterior aspect of the skull shows precisely these conditions. These skulls are, as Watson points out, high in proportion to width, "square-cut" in transverse section, and flat-cheeked, more like pelycosaurs than like *Captorhinus* in these respects. The proportions of the occipital aspect of Watson's restoration of *Protorothyris* are very nearly the same as those of *Dimetrodon*. The quadrate lies well below the level of the otic capsule, and although the stapes is not shown, it must have slanted downward quite steeply to reach the stapedial recess on the back of the quadrate (fig. 6, D). The precise nature of the dorsum of the stapes is not known in either *Protorothyris* or *Romeria,* but Watson states that the stapes of a similar captorhinomorph from West Virginia, *Melanothyris morani,* "in effect shows a modification in shape analogous to that of the immensely larger stapes of *Ophiacodon.*" *Protorothyris* has no retroarticular process, and the lower jaw of *Romeria* is unknown. It is probable that the tympanum was attached in these forms as it was in the large pelycosaurs. The series *Protorothyris-Romeria-Captorhinus* shows in diagrammatic fashion the progressive raising of the level of the quadrate.

The ear region of the small pelycosaurs underwent a series of changes similar to those followed by the captorhinomorphs. However primitive they may be in other respects, the small pelycosaurs are more advanced in the relationship between otic capsule, quadrate, and stapes than are

larger members of the order, just as *Captorhinus* is more advanced in this respect than is *Protorothyris*. In no small pelycosaur is the quadrate known certainly to lie below the level of the otic capsule, but one is tempted to seek (or postulate) a small, deep-cheeked form with a middle ear from which that of both the large and small pelycosaurs could have been derived. As Romer suggests, such an animal would be very similar to *Protorothyris* in size and general shape of skull. *Protorothyris* itself is disqualified from a position at the base of the pelycosaur line by such features as reduction of the tabular bone, in which it has already begun to specialize toward a captorhinomorph rather than a pelycosaur condition of the skull.

An alternative possibility is that the basal pelycosaurs were not small, but moderate to large animals. The fact that the pelycosaurs as a group are rather large animals for their time (Romer and Price, 1940) supports this idea. *Limnoscelis*, a very primitive captorhinomorph, is also moderately large, as is *Anthracosaurus*, an amphibian close to the stem of both the captorhinomorph and pelycosaur lines. Romer (p. 197, 1940) points out that no really primitive pelycosaur is known, in the sense of an animal representing the basal stock of the order. The ophiacodonts are close to the base, however, and if it be accepted that the basal type was a fairly large animal, the middle ear of *Ophiacodon* itself may well represent as primitive condition of the region as will be found in the pelycosaurs.

The pelycosaurs which remained moderately large, or evolved toward a larger size, tended to maintain or increase the primitive depth of the quadrate region, perhaps in response to new methods of feeding. Watson (1953) suggests that deepening took place in the sphenacodonts in response to the increased size of the anterior maxillary teeth, which required a wider gape. In the large ophiacodonts on the other hand, high anterior teeth were not developed; maintenance of a deep quadrate in these forms may have resulted from a need for a larger mass of trigeminal musculature, brought about by increased activity or merely by increase in size. The relatively primitive quadrate depth in *Edaphosaurus* may also have been maintained to allow room for large trigeminal musculature evolved in response to plant feeding. Presumably the feeding habits of the small pelycosaurs were enough different, perhaps in part because of different predator-prey size relationships, that instead of maintaining the jaw articulation at a primitive depth or forcing it to deepen, selection forced it to become shallower.

The selective forces, then, which wrought the changes in the middle ear from amphibian to reptile were those which acted upon the jaw mechanism rather than upon the middle ear itself. The backward movement of the amphibian quadrate forced the tympanum out of the otic notch and into direct support by the stapes. This stage was retained in the large pelycosaurs because their feeding habits caused them to retain or exaggerate the depth of the jaw articulation. Changes of head shape and jaw structure raised the jaw articulation in the small pelycosaurs and also in the captorhinomorphs, which in turn moved the stapes into its horizontal position and forced the tympanum to lie terminal to the stapes. Through all these changes of skull and jaws, the middle ear retained the function for which it was originally evolved by amphibians, detection of air-borne sound by means of a membrane. Changes in the ear were merely reflections of changes occurring in other structures, the ear making do at any stage, so to speak, with what it had at the time.

Summary

The theory, propounded chiefly by Watson, that the middle ear in pelycosaur and captorhinomorph reptiles lacked a tympanum, is based upon the ideas that the stapes is too large and too firmly attached to various surrounding bones to be moved

bodily by a membrane of reasonable size, and that the back of the skull is not of the proper shape to support a tympanum terminal to the stapes. These concepts are challenged as follows:

1. It is pointed out that the amplitude of stapedial oscillation in the mammals is extremely minute, and that the ligamentous or cartilaginous attachments of the pelycosaur stapes would in all probability have permitted movement of the same order of magnitude.

2. A series of experiments with working models representing arrangements possible in the pelycosaur middle ear demonstrates that a bone the size of the pelycosaur stapes will transmit air-borne sound picked up by a membrane of the order of only $\frac{1}{23}$ to $\frac{1}{14}$ of its mass. Membranes bearing this relationship to known *Ophiacodon* and *Dimetrodon* stapes need be from no more than about 12 mm to about 30 mm in diameter, depending on the size of the stapes. This is quite small enough to fit behind the pelycosaur quadrate, terminal to the stapes. The experiments also demonstrate that bones the size of the pelycosaur stapes transmit sound more effectively by bodily movement than by molecular movement.

3. The shape and orientation of the stapes, and a crest on its dorso-lateral surface, suggest that the tympanum was attached by its medial margin to the shaft and dorsal process of the stapes in the larger pelycosaurs, rather than receiving a terminal tympanic process on its surface in more "normal" reptilian fashion. The lateral margin of tympanum was attached to squamosal. A very large tympanum would fit the pelycosaur skull in this position. Articulation of the dorsal process suggests that the stapes of *Dimetrodon,* at least, was specifically adapted to be driven by a tympanum attached along its shaft.

Support of a tympanum as advocated in item "3" bears the following evolutionary implications:

1. This position is a logical consequence of the mode of closure of the otic notch in transition from the amphibian type of middle ear. The dorsal support of the amphibian tympanum is brought very close to the dorsal process of the stapes during closure of the notch. As the notch closes, part of the old support of the tympanum is lost but is immediately replaced by the stapes itself; in providing this support the downward-slanting, ligamentous internal process of the amphibian is ossified to become the downward-slanting shaft of the pelycosaur stapes. It is suggested that the large size of the pelycosaur stapes is a direct result of its support of a tympanum in this manner.

2. The horizontal attitude of the stapes in the small pelycosaurs and in *Captorhinus* more nearly resembles the "orthodox" reptilian position. However, attachments of the stapes to surrounding bones are the same in these forms as in the large pelycosaurs, and it is suggested that stapes position in the large forms is more primitive, the horizontal attitude being derived from it by a raising of the level of the quadrate, which in turn forced the membrane to lie terminal to the stapes. *Protorothyris, Romeria,* and *Captorhinus,* as restored by Watson, demonstrate the raising of the level of the quadrate in the captorhinomorphs. The middle ear of *Ophiacodon* probably represents the most primitive condition of the region in the pelycosaurs.

3. All of the changes in the middle ear from the amphibian condition to that of *Captorhinus* and the small pelycosaurs appear to be the result of changes brought about by selection acting on the jaw or jaw suspension, rather than directly on the middle ear. The ear arose among the amphibians as a mechanism which functioned to detect air-borne sound by means of a membrane, and so it remained throughout the transition to reptiles, making do with what it had at each stage.

Acknowledgments

The writer wishes to express his thanks to Professor A. S. Romer of the Museum of Comparative Zoology, Harvard, for the use of casts of occiput and stapes of *Dimetrodon,* and stapes of *Ophiacodon*. Thanks are also due to Dr. F. R. Parrington of the University Museum of Zoology, Cambridge (England), and to Professor Romer for the encouraging interest they have shown in the experimental part of this work.

Literature Cited

Bekesy, G. von, and W. A. Rosenblith. 1951. The mechanical properties of the ear. In: Handbook of experimental psychology, S. S. Stevens, Ed. John Wiley and Sons, Inc., New York.

Gregory, W. K. 1910. The orders of mammals. Bull. Amer. Mus. Nat. Hist., **27**: 1–524.

Oelrich, T. M. 1956. The anatomy of the head of *Ctenosaura pectinata*. Miscel. Publ. Museum of Zoology, Univ. of Michigan, **94**.

Parrington, F. R. 1946. On the cranial anatomy of cynodonts. Proc. Zool. Soc. London, **116**: 181–197.

——. 1949. Remarks on a theory of the evolution of the tetrapod middle ear. Jour. Laryngology and Otology, **63**: 580–585.

——. 1955. On the cranial anatomy of some gorgonopsids and the synapsid middle ear. Proc. Zool. Soc. London, **125**: 1–40.

Price, L. I. 1935. Notes on the braincase of *Captorhinus*. Proc. Boston Soc. Nat. Hist., **40**: 377–386.

Romer, A. S., and L. I .Price. 1940. Review of the Pelycosauria. Geol. Soc. America Special Papers **28**.

Tumarkin, A. 1948. The evolution of the auditory conducting apparatus. A new synthesis based on functional considerations. Proc. Roy. Soc. Med., **41**: 877–888.

——. 1949. On the evolution of the auditory conducting apparatus. Jour. Laryngology and Otology, **63**: 119–140, 193–216.

——. 1955. On the evolution of the auditory conducting apparatus: a new theory based on functional considerations. Evolution, **9**: 221–243.

Watson, D. M. S. 1926. The evolution and origin of the amphibia. Phil. Trans. Roy. Soc. London (B), **214**: 189–257.

——. 1948. *Dicynodon* and its allies. Proc. Zool. Soc. London, **118**: 823–877.

——. 1951. Paleontology and modern biology. Yale Univ. Press, New Haven.

——. 1953. Evolution of the mammalian ear. Evolution, **7**: 159–177.

——. 1954. On *Bolosaurus* and the origin and classification of reptiles. Bull. Mus. Comp. Zool., **111**: 297–449.

Westoll, T. S. 1943. The hyomandibular of *Eusthenopteron* and the tetrapod middle ear. Proc. Roy. Soc. London (B), **131**: 393–414.

Wever, E. G., and J. A. Vernon. 1956. Sound transmission in the turtle's ear. Proc. Nat. Acad. Sci., **42**: 292–299.

Vaughn, P. P. 1955. The Permian reptile *Areoscelis* restudied. Bull. Mus. Comp. Zool., **113**: 305–467.

ADAPTIVE SHIFT AND DISPERSAL IN A TROPICAL ANT FAUNA

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Received April 16, 1958

Introduction

The tropics have long been recognized as the site of maximum evolutionary activity on land. In tropical rain forests, evolution proceeds simultaneously in the largest number of species. A growing amount of evidence of diverse kinds also points to the rain forests, particularly those of continents and the large islands, as the "center" of evolution of a majority of major animal and plant groups, where these groups have diversified maximally in various stages of their phyletic history and out of which they have tended to spread into adjacent temperate and arid regions (Richards, 1952; Darlington, 1957).

According to the theory stressed by Darlington it is the Old World tropics specifically that form the principal evolutionary center of the vertebrates. Successful groups, achieving "general adaptation," send emigrant species out of the Old World tropics into temperate zones, where they may diversify secondarily and in time come to show zonation and radial dispersal of their own. From time to time faunal drift occurs across the region of the present-day Bering Straits. Movement across this barrier has been predominantly out of the Old World into the New. Once emigrant species reach the New World, further diversification may take place; this becomes especially probable if members of the group succeed in penetrating the Neotropical forests.

The nature of general adaptation and the dispersal mechanisms underlying major biotic movement is clearly one of the great problems of modern evolutionary theory. It appears that our knowledge has now reached the stage where finer analyses of these causal processes can and should be undertaken. There is a need for a "biogeography of the species," oriented with respect to the broad background of biogeographic theory but drawn at the species level and correlated with studies on ecology, speciation, and genetics. In the present paper one such analysis is attempted in very preliminary form, dealing with the ponerine ants [1] of Melanesia. This fauna is unusually suitable for a study of the kind proposed. Melanesia, including New Guinea, has proven to be a peripheral or "recipient" zoogeographic and for ants, i.e., most of the present fauna has been derived ultimately from immigrations from southeastern Asia and Australia, while proportionately few Melanesian-centered groups have emigrated into these adjacent source areas. Hence dominant, successful groups can conceivably be distinguished in their early stages of expansion as they first enter Melanesia, and older resident elements can be studied to piece together details of the later history of invading groups. Theoretically, it should then be possible to characterize the early invading groups ecologically and to infer some of the attributes that have contributed to their successful dispersal, in short, the "attributes of success" associated with general adaptation.

Patterns of Speciation

Taxonomic analysis of the Melanesian ponerine fauna has revealed a remarkably

[1] The subfamily Ponerinae is one of eight subfamilies of ants (Formicidae) known from Melanesia. The species known constitute approximately one-fifth of the described ant fauna. Revisionary work on the Melanesian ants has been conducted at Harvard University during the past three years and is now virtually complete (Wilson, 1957 et seq.; Brown, 1958; Willey and Brown, ms.).

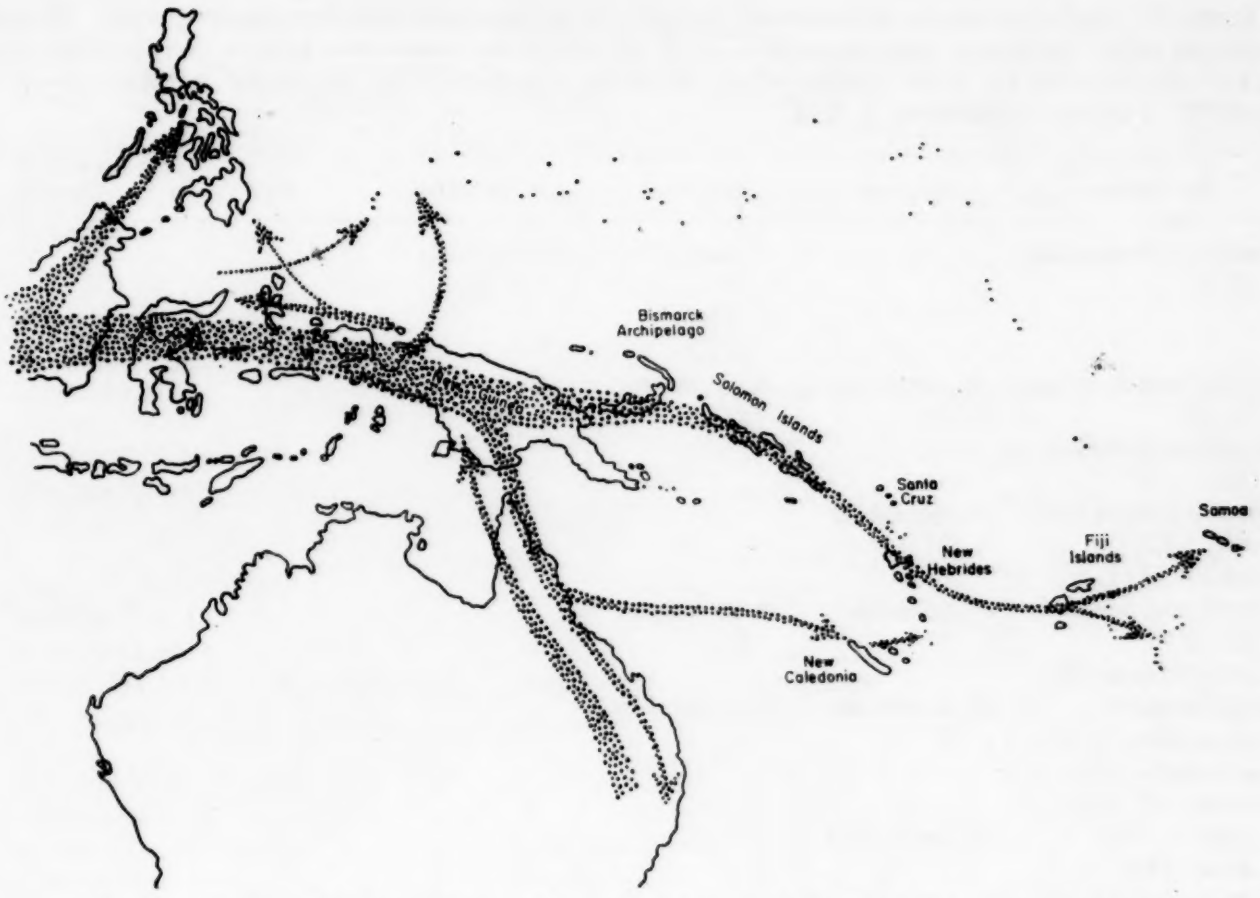


FIG. 1. Routes of dispersal in and out of Melanesia followed by the ponerine ants.

consistent order of zoogeographic patterns. These are summarized in the sections below.

(1) Most groups seem to invade New Guinea from southeastern Asia. Many of these also reach tropical Queensland, and a substantial part of the Queensland fauna is thus composed of Oriental stocks held in common with New Guinea. A few old Australian groups, lacking any apparent affinity with the Oriental fauna, also invade New Guinea. Occasionally, endemic Papuan groups move back in the direction of southeastern Asia but rarely if ever reach beyond the Moluccas and Philippines (fig. 1).

(2) From New Guinea a fraction of the invading stocks presses on to outer Melanesia. An ever-diminishing number reaches the Bismarck Archipelago, Solomon Islands, New Hebrides, and Fiji Islands. Progress along this route follows the classical "filter" effect that applies generally to the faunas of archipelagic chains with permanent water gaps. Some secondary radiation occurs in the various island groups, especially the Fiji Islands, but there is at present no concrete evidence indicating any reverse movement of these precinctive species back toward New Guinea (fig. 1, table 1).

(3) Because radiation has been more extensive on the Fiji Islands, the fauna of this archipelago appears to be larger than that of the adjacent New Hebrides. However, this is true only in the sense that the total number of known species is larger. When species groups are counted instead of species, the fauna of the New Hebrides proves to be somewhat the larger. Since the number of species groups corresponds more closely to the actual number of original immigrant species, this estimate is to be considered a truer measure of relative accessibility of archipelagos to the mainland fauna (fig. 11, table 1).

(4) New Caledonia is faunistically very distinct from the remainder of Melanesia. Despite its proximity to the New Hebrides and Fiji Islands, it does not appear to have received any of its ponerine fauna

TABLE 1. *List of the species of Ponerinae known from Melanesia, broken into species groups.* Roman numerals after the names indicate evolutionary classification; lower case letters (a–f) indicate ecological distribution on New Guinea alone, referring specifically to the major habitats given in figure 9. Further explanation in text.

New Guinea	Bismarcks	Solomons	N. Hebrides	Fijis	Samoa
Amblyopone australis (I, f)		*A. australis* (I) *A. celata* (III)	*A. australis* (I)		
Myopopone castanea (I, ef)	*M. castanea* (I)	*M. castanea* (I)			
Mystrium camillae (I)					
Prionopelta majuscula (III)	*P. majuscula* (III)				
P. opaca (? II, ef)					
Platythyrea parallela (I, cdf)	*P. parallela* (I)	*P. parallela* (I)			*P. parallela* (I)
P. quadridenta (II)					
Rhytidoponera araneoides (I, bc)	*R. araneoides* (I)	*R. araneoides* (I)			
R. celtinodis (III)					
R. inops (II, def)					
R. gagates (III)	*R. nexa* (III)				
R. nexa (III)					
R. purpurea (II, f)					
R. abdominalis (III, f)					
R. aenescens (III)					
R. laciniosa (III, ef)					
R. rotundiceps (III, f)					
R. strigosa (III, ef)					
R. subcyanea (III, e)					
Gnamptogenys biroi (II, f)					
G. grammodes (II, f)					
G. macretes (II, f)					
G. major (II, f)					
G. cribrata (II, e)		*G. malaensis* (II)			
G. epinotalis (II, f)		*G. albiclava* (III)			
		G. crenaticeps (III)			*G. aterrima* (III)
		G. lucida (III)			
Proceratium papuanum (II, f)					*P. relictum* (III)
Discothyrea clavicornis (II, f)		*D. clavicornis* (II)			
Ponera biroi (III, ef)					
P. macradelphe (III, f)	*P. biroi* (III)	*P. biroi* (III)			*P. eutrepta* (III)
P. punctiventris (III, f)		*P. sororcula* (III)			*P. turaga* (III)
P. sororcula (III, cd)					
P. confinis (I, ef)	*P. confinis*	*P. confinis*	*P. confinis*		
P. pallidula (II, ef)	(I)	(I)	(I)		

TABLE 1—*Continued*

New Guinea	Bismarcks	Solomons	N. Hebrides	Fijis	Samoa
P. papuana (III)					
P. pruinosa (I, cdef)		*P. pruinosa* (I)	*P. pruinosa* (I)	*P. monticola* (III)	
P. sabronae (III, f)				*P. vitiensis* (III)	
P. clavicornis (I, def)					
P. elegantula (III, f)					
P. selenophora (III, e)		*P. clavicornis* (I)	*P. clavicornis* (I)	*P. colaensis* (III)	
P. syscena (III, f)					
P. xenagos (III, f)					
		P. gleadowi (I)			*P. gleadowi* (I)
P. tenella (III, f)					
P. huonica (III, f)					
P. petila (III, e)					
P. szaboi (III, e)	*P. ratardorum* (III)	*P. ratardorum* (III)	*P. ratardorum* (III)		
P. szentivanyi (III, e)					
P. tenuis (III, f)					
Brachyponera arcuata (I)		*B. croceicornis* (I)			
B. croceicornis (I, cdef)					
Mesoponera manni (I, f)		*M. manni* (I)			
M. papuana (II, e)					
Trachymesopus crassicornis (III, f)		*T. crassicornis* (III)			
		T. sheldoni (III)			
T. darwini (I)		*T. darwini* (I)	*T. darwini* (I)		
T. stigma (I, cdef)	*T. stigma* (I)	*T. stigma* (I)	*T. stigma* (I)	*T. stigma* (I)	*T. stigma* (I)
Ectomomyrmex aciculatus (II, e)		*E. acutus* (II)			
E. acutus (II)	*E. acutus* (II)	*E. aequalis* (II)			
E. exaratus (II, e)					
E. scobinus (II, e)					
E. simillimus (II, e)					
E. striatulus (II, ef)					
					E. insulanus (II)
Bothroponera incisus (?)					
B. obesus (?)					
Cryptopone butteli (I, ef)	*C. butteli* (I)				
C. fusciceps (II, ef)		*C. fusciceps* (II)			
C. testacea (I, e)		*C. testacea* (I)			
C. motschulskyi (III, def)					
Diacamma rugosum (I, cdef)					

TABLE 1—*Continued*

New Guinea	Bismarcks	Solomons	N. Hebrides	Fijis	Samoa
Myopias cribriceps (III, e)					
M. delta (III, e)					
M. concava (III, ef)					
M. foveolata (III)					
M. gigas (III, e)					
M. julivora (III, e)					
M. levigata (III, f)					
M. loriai (III, f)					
M. media (III, f)					
M. ruthae (III, e)					
M. xiphias (III)					
M. latinoda (II, f)					
M. tenuis (?III, cdef)					
M. tylion (?III, ef)					
Leptogenys bituberculata (II, ef)				*L. foveopunctata* (II)	
L. drepanon (II)			*L. hebrideana* (II)	*L. fugax* (II)	
L. indagatrix (II, f)				*L. humiliata* (II)	
L. papuana (II)				*L. letilae* (II)	
L. triloba (III, f)				*L. navua* (II)	
				L. vitiensis (II)	
L. breviceps (II, ef)					
L. caeciliae (III, f)					
L. optica (III, ef)					
L. diminuta (I, cdef)	*L. diminuta* (I)	*L. diminuta* (I)			
L. nitens (II)		*L. oresbia* (II)			
L. purpurea (II, f)					
L. foreli (I)	*L. emeryi* (III)	*L. foreli* (I)	*L. foreli* (I)		
		L. truncata (III)			
L. keysseri (III, f)					
Odontomachus latissimus (II)					
O. linae (II, f)					
O. malignus (I, a)					
O. imperator (II)		*O. emeryi* (II)			
O. montanus (II)	*O. malignus* (I)	*O. malignus* (I)		*O. angulatus* (II)	
O. opaculus (II, f)					
O. papuanus (I, ef)					
O. rufithorax (II)					
O. saevissimus (I)					
O. tauerni (II)					
O. aciculatus (III)					
O. aeneus (III)	*O. simillimus* (I)	*O. simillimus* (I)	*O. simillimus* (I)	*O. simillimus* (I)	*O. simillimus* (I)
O. cephalotes (I, ef)					
O. simillimus (I, bcdf)					
O. nigriceps (III, e)					
O. testaceus (III, ef)	*O. tyrannicus* (III)				
O. tyrannicus (III, f)					
Anochetus cato (III, f)	*A. cato* (III)	*A. cato* (III)			
		A. isolatus (III)			

TABLE 1—*Continued*

New Guinea	Bismarcks	Solomons	N. Hebrides	Fijis	Samoa
A. chirichinii (III, ce) *A. fricatus* (III, e) *A. graeffei* (I, d) *A. variegatus* (III) *A. vesperus* (III, d)	*A. graeffei* (I)	*A. graeffei* (I)	*A. graeffei* (I)	*A. graeffei* (I)	*A. graeffei* (I)

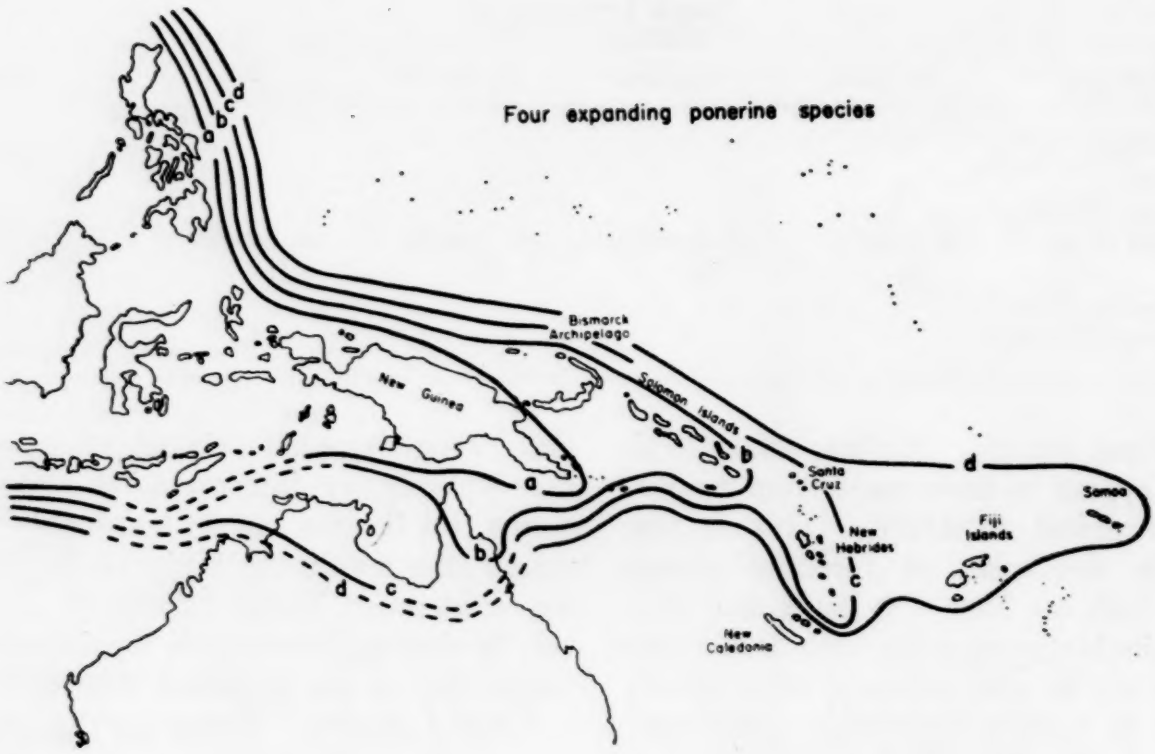
from these islands. Rather, all endemic stocks appear to have come from tropical or subtropical Australia. This is true even in the cases of Oriental groups found both on New Caledonia and elsewhere in Melanesia; the New Caledonian species are in each instance most closely related to eastern Australian representatives of the invading Oriental group. Thus Oriental groups reaching New Caledonia, of which there are a goodly number, appear to have entered by way of eastern Australia. One species (*Amblyopone australis*) has spread from New Caledonia to the southern New Hebrides, thus providing a slender link between these two segments of the Melanesian fauna. The New Caledonian endemics show nearly every conceivable degree of evolutionary divergence from the cognate Australian species, from a clearly conspecific condition to a highly modified form approaching a distinct generic level (figs. 1, 4, 5, 6).

(5) Individual ponerine species and species-groups in Melanesia seem to show various steps in a sequence of expansion, diversification, and contraction. In fact, when the distributions and phylogenetic positions of all of the species are considered as a whole, the conclusion is almost inescapable that such a process is nearly universal in this group of ants. The steps are illustrated by the actual distributions of species shown in figures 2–8. These examples are admittedly selected, and the evolutionary history they are supposed to follow hypothetical. Yet virtually all of the patterns of the species groups fit somewhere within this unilateral zoogeographic classification, and the evolutionary interpretation proposed seems to be the most reasonable and simple one within the limits of the existing evidence. Three classes of species can be distinguished, each representing a major step in the historical sequence.

Stage-I species. These are the species apparently in the process of expansion, either from southeastern Asia or Australia into Melanesia, or out of Melanesia back into the primary source areas, or out of New Guinea through most of the remainder of Melanesia. Thus two categories of Stage-I species are hypothesized: *primary,* including Oriental- or Australian-based species invading Melanesia (as well as Oriental-based species invading New Guinea and then Australia by way of New Guinea); and *secondary,* including Papuan species that are pushing well out into outer Melanesia or invading southeastern Asia or Australia. Stage-I species generally have continuous ranges and show little geographic variation, although non-geographic variation may be considerable (fig. 2).

Stage-II species. These are species which have differentiated to species level in Melanesia, i.e., are Melanesian precinctives, but which belong to species groups centered outside Melanesia, either in southeastern Asia or Australia. They represent stocks which are relatively recent invaders but which have been resident sufficiently long to diverge to species level (Figs. 3, 4).

Stage-III species. These are Melanesian precinctives belonging to Melanesian-centered species groups. That such



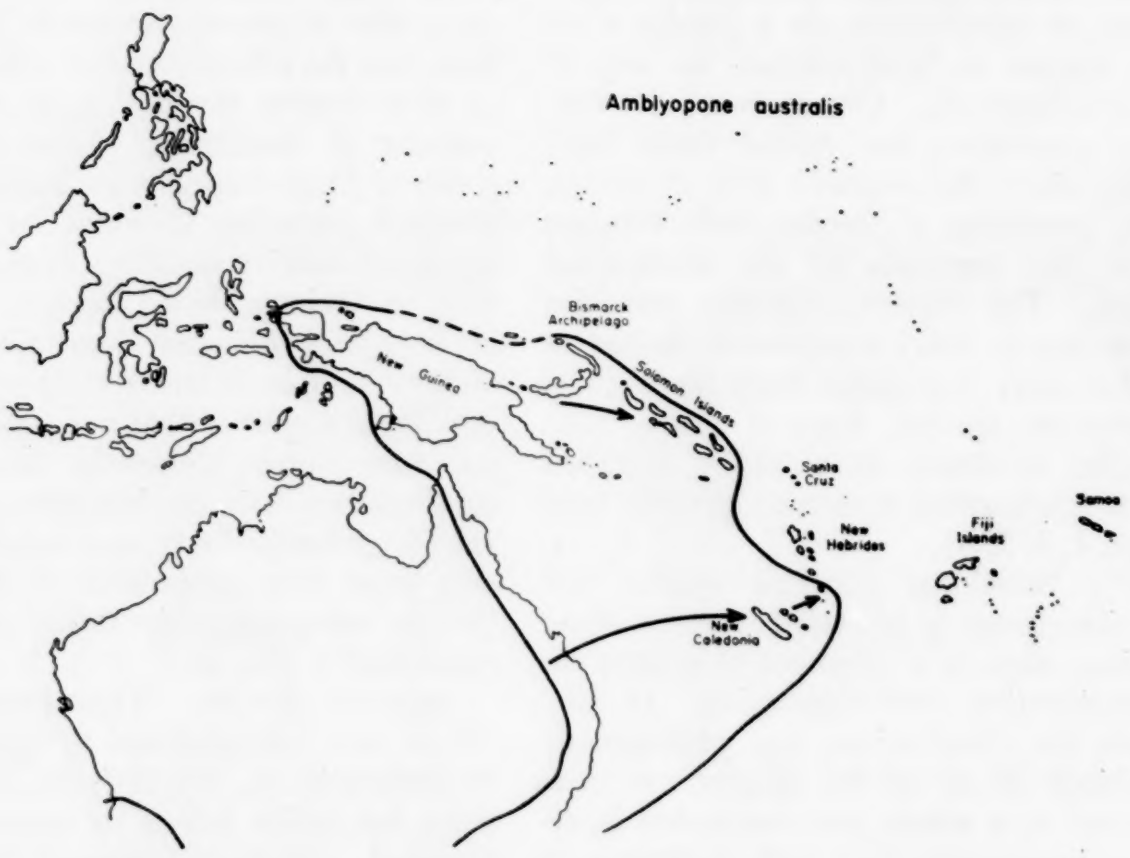


FIG. 2. Maximum known distributions of several Stage-I ponerine species. Upper: four unrelated species believed to represent successive stages of invasion from Asia; *a, Diacamma rugosum; b, Myopopone castanea; c, Trachymesopus darwini; d, T. stigma.* Lower: the range of *Amblyopone australis,* which has entered Melanesia from Australia in two places; a double entry is inferred from the fact that the Papuan-Solomons and New Caledonian-New Hebridean populations are marked by opposing trends in geographic variation.

In this and subsequent distribution maps the following qualifications should be noted. The precise limits of the species ranges in the Philippines, lesser Sundas, and lesser island groups such as the Louisiade and Manus Archipelagos are unknown. Dashed lines indi-

groups are ultimately Oriental or Australian in origin is suggested by the fact that groups in stages intermediate between II and III exist (e.g., groups of *Ponera selenophora* and *P. tenuis*). These have a few members with relict distributions outside of Melanesia that appear (within the framework of the present hypothesis) to be the last remnants of a contracting central (Asian or Australian) fauna (figs. 5–8).

Most Stage-III species have sufficient potency to continue diversifying, and some are able to expand out of Melanesia again, thus secondarily achieving Stage-I status (figs. 6, 7, 8, 12). Eventually, however, they probably enter upon a phase of irrevocable recession, their ranges contracting more and more until they can properly be labeled "relict" species (fig. 8). Presumably the next and final step for most is extinction, or extreme specialization, perhaps hastened by the inroads of freshly competing Stage-I and Stage-II species.

Ecological Attributes of the Expanding (Stage-I) Species

Using the zoogeographic criteria outlined in the preceding section, it is now possible to make a qualified distinction of those species that are in the early stages of expansion in or out of Melanesia. In the great majority of cases these belong to "dominant" Oriental- or Australian-centered species groups, i.e., groups that are relatively abundant, widespread, and diversified in the source areas. When ecological data gathered by the author during recent field work in New Guinea was analyzed, it was shown that Stage-I species in this area are characterized by (1) a proportionately greater concentration in marginal habitats, marginal in this case being defined specifically as open lowland forest, grassland, and littoral[2] (table 2, fig. 9) and (2) a greater ecological amplitude (table 3, fig. 10). Further, when the composition of the Melanesian fauna is broken down according to successive archipelagos, it was shown that a significantly larger proportion of Stage-I species occupies central Melanesia than is the case in New Guinea and the Fiji Islands (table 4, fig. 11). The evolutionary implications of these interesting correlations will now be considered.

Discussion

If the zoogeographic sequence postulated here is correct, it seems to lead to the conclusion that ponerine species invade New Guinea, and outer Melanesia through New Guinea, by way of the relatively sparsely populated marginal habitats. When occasional Melanesian-based species succeed in expanding out of New Guinea into the Philippines or Australia, they apparently leave by the same adaptive route. This ecological attribute is the most significant one evident within the limits of the existing data.

It should be mentioned that there does not seem to be any other peculiarity in mode of dispersal or nest-site preference in Stage-I species that can account for their current expansion. These species include *Diacamma rugosum* and *Leptogenys foreli,* which have wingless queens, as well as many species that are presumed

[2] The central habitats are the "inner" rain forest, including denser lowland forest and montane (mid mountain) forest up to approximately 1000 meters elevation. Marginal habitats also have the smallest ponerine faunas in terms of absolute numbers of species.

cate that the presence of the species anywhere in the island group under question is not known but believed likely. In most cases the precise limits of the ranges within larger archipelagos, such as the Solomon Islands and New Hebrides, are not known; where the entire archipelago is enclosed this can be taken certainly to mean only that the species occurs somewhere within the archipelago. Detailed locality data can be found in the revisionary basis of this article published elsewhere and cited in the bibliography.

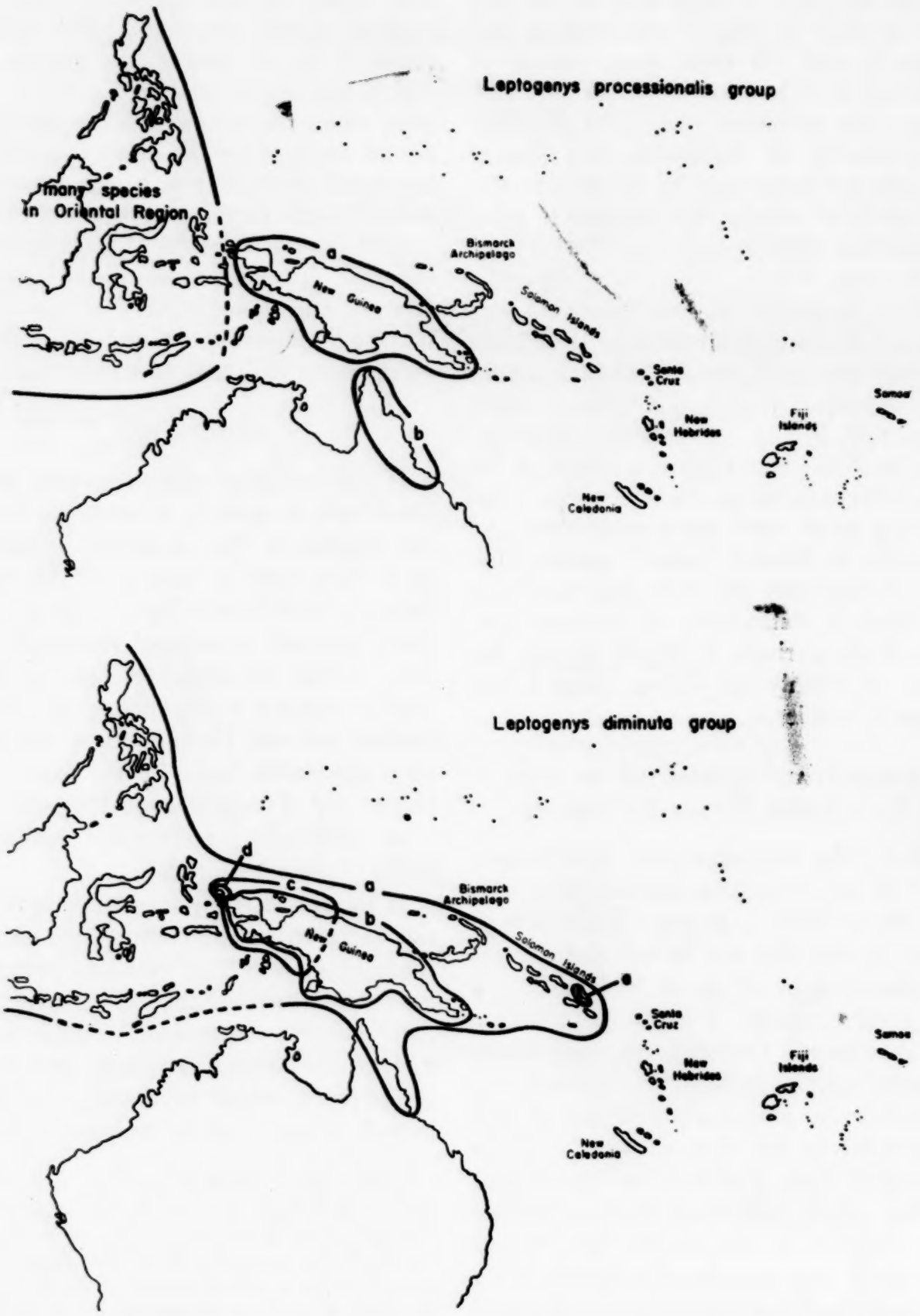
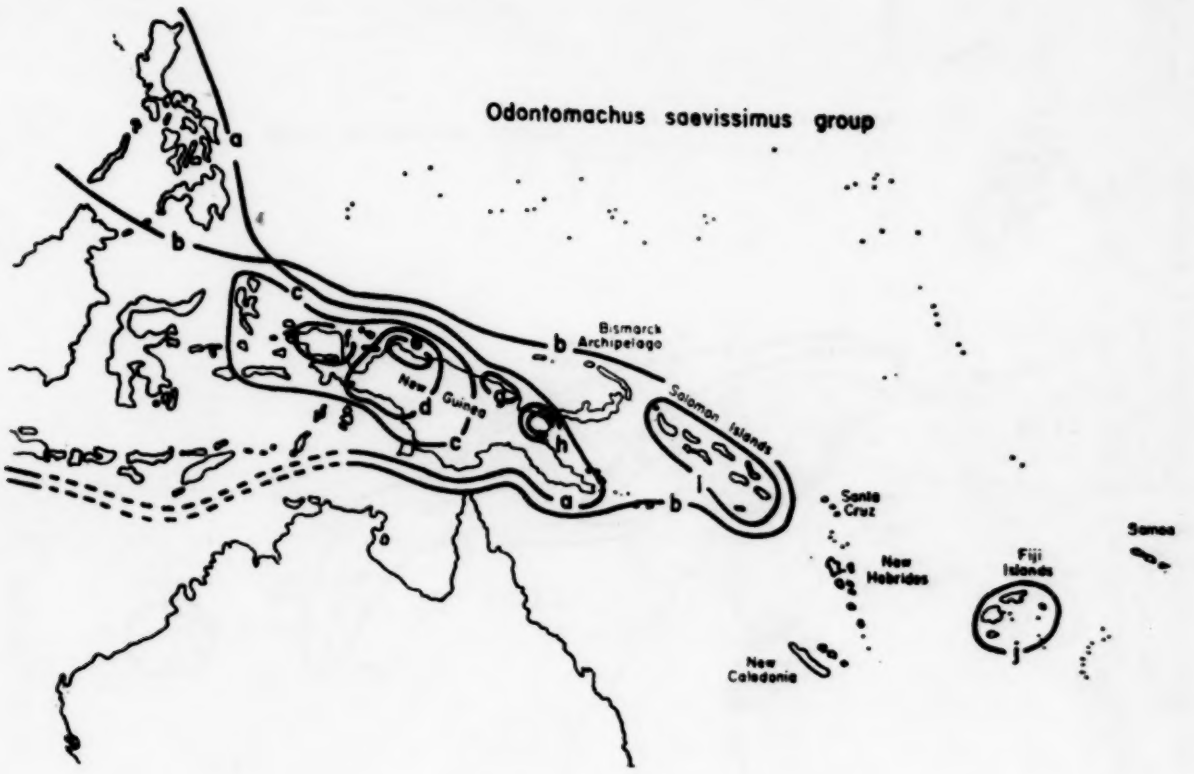


FIG. 3. Maximum known distributions of species in early Stage II. Upper: *a, Leptogenys breviceps; b, L. fallax.* Lower: *a, L. diminuta; b, L. purpurea; c, L. nitens; d, L. violacea; e, L. oresbia.*



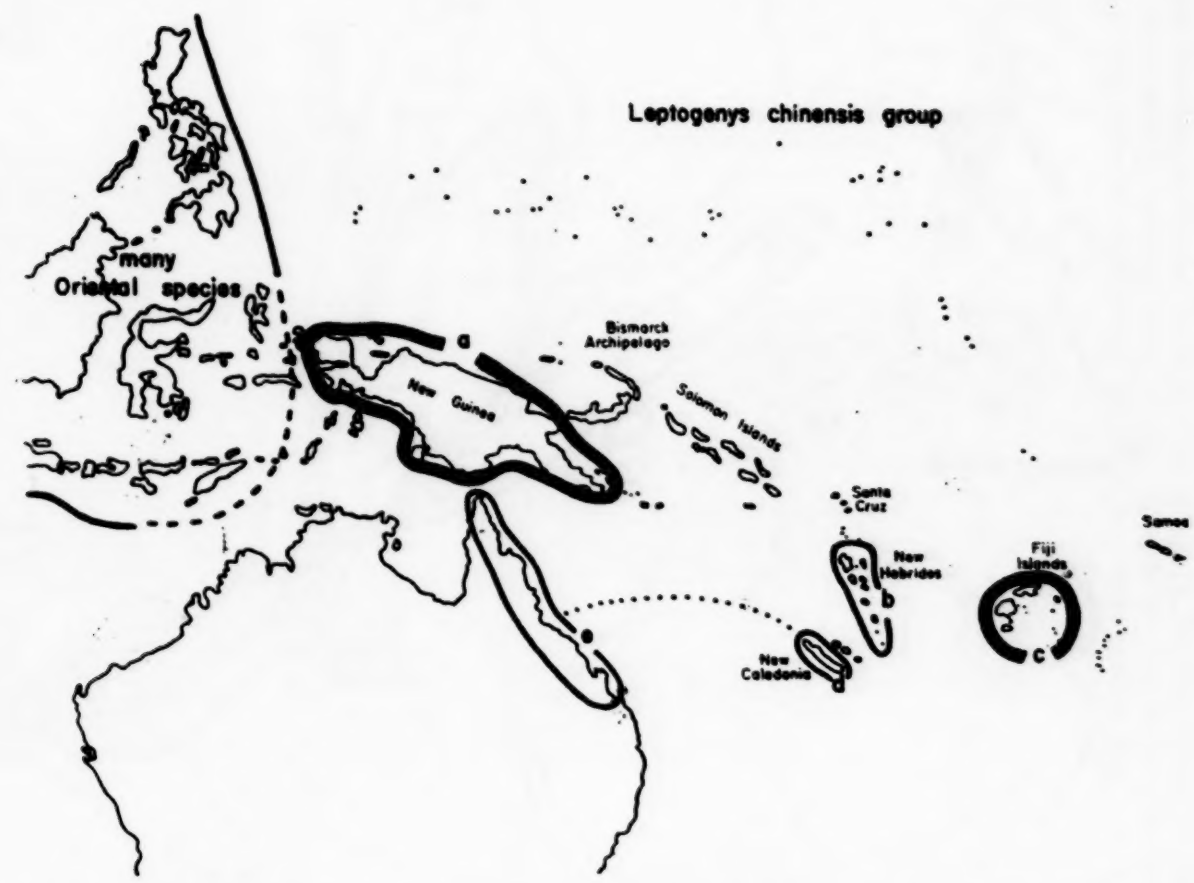


FIG. 4. Maximum known distribution of species in advanced Stage II. Upper: *a, Odontomachus rixosus; b, O. malignus; c, O. tauerni; d, O. rufithorax; e, O. linae; f, O. imperator; g, O. opaculus; h, O. latissimus* and *O. montanus; i, O. emeryi; j, O. angulatus.* Lower: *a, Leptogenys bituberculata; L. drepanon, L. indagatrix, L. papuana, L. triloba* (the precise limits of these species on New Guinea is not indicated); *b, L. hebrideana; c, L. foveopunctata; L. fugax, L. humiliata, L. letilae, L. navua, L. vitiensis; d, L. sagaris; e, L. anitae.* The dotted line suggests the close relationship of the New Caledonian *sagaris* and Australian *anitae.*

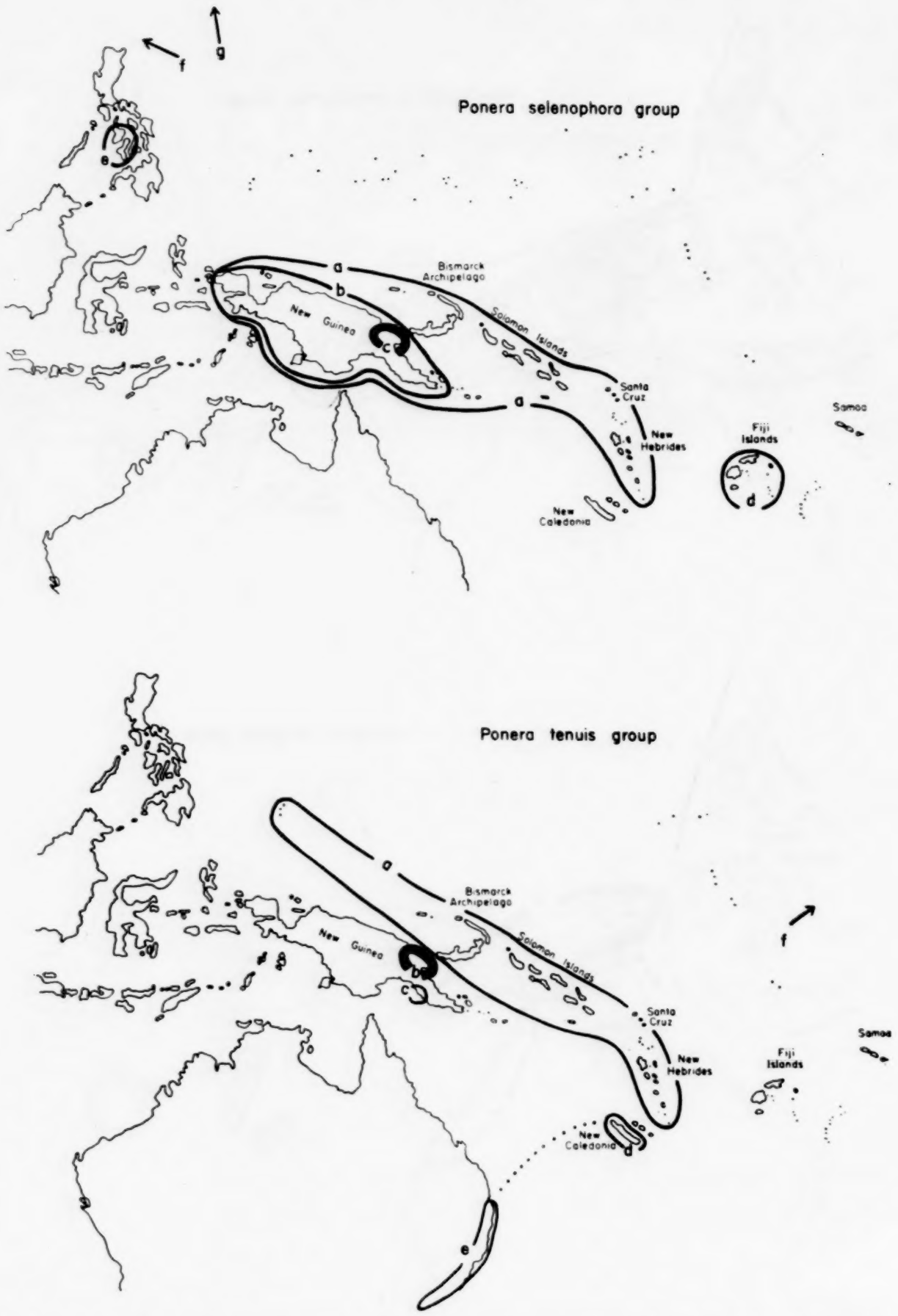


FIG. 5. Maximum known distribution of species in early Stage III, in which the Oriental source fauna has contracted and at present contains only a few relict species. Upper: *a*, *Ponera clavicornis* (ranked as a secondary Stage-I species); *b*, *P. selenophora*; *c*, *P. elegantula*, *P. syscena*, *P. xenagos*; *d*, *P. colaensis*; *e*, *P. oreas* (Philippines); *f*, *P. sinensis* (Hong Kong); *g*, *P. scabra* (Honshu). Lower: *a*, *P. ratardorum*, *b*, *P. huonica*, *P. petila*, *P. szaboi*, *P. tenuis*; *c*, *P. szentivanyi*; *d*, *P. caledonica*; *e*, *P. exedra* (Australia); *f*, *P. swezeyi* and *P. zwaluwenburgi* (Hawaii); *g*, *P. incerta* (Java). The dotted line suggests the close relationship between the New Caledonian *caledonica* and Australian *exedra*.

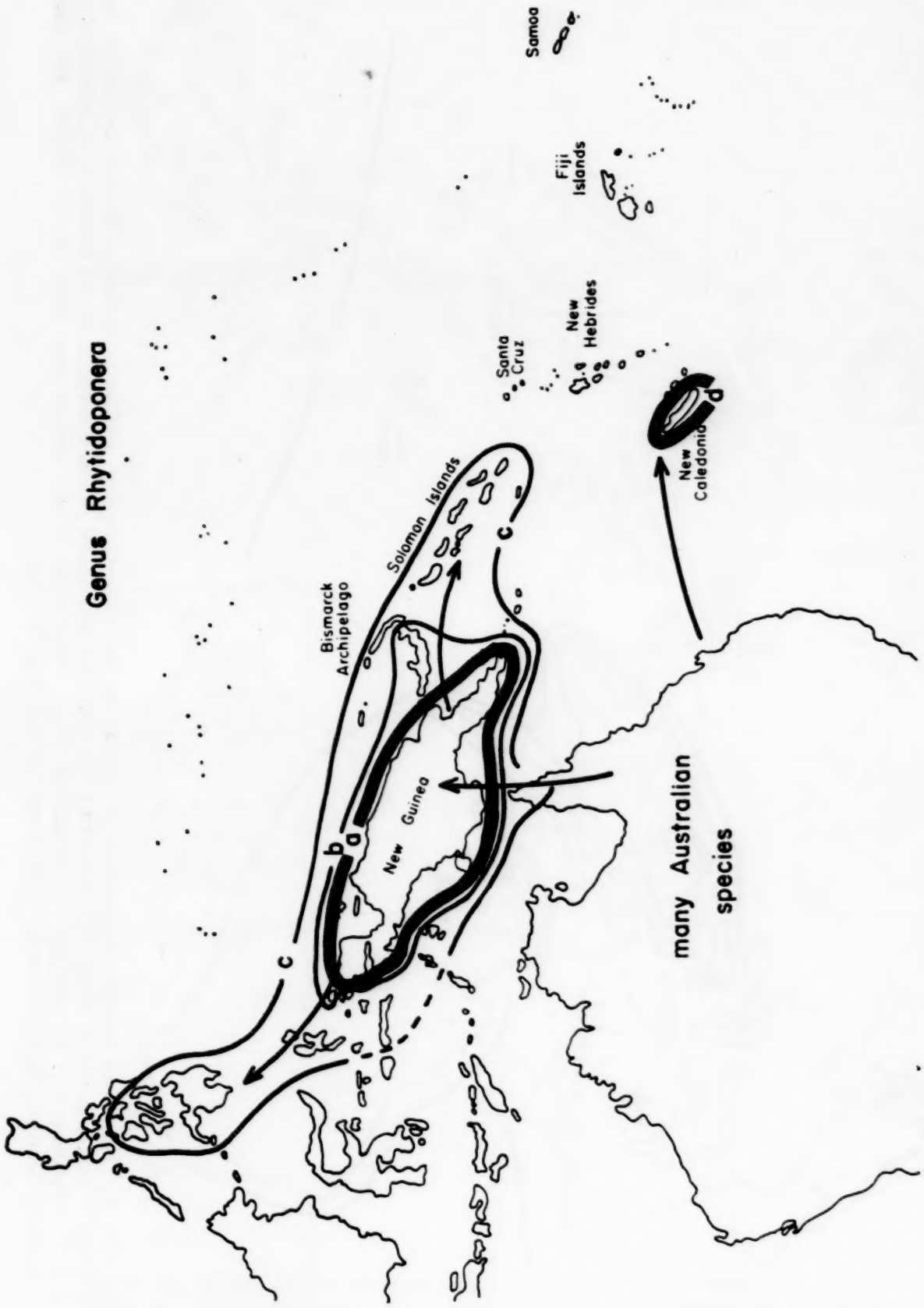


FIG. 6. The distribution of the genus *Rhytidoponera*, which is ultimately Australian in origin but which is differentiating and expanding secondarily out of New Guinea. Most of the Papuan species belong to Papuan-centered species groups and are classified in Stage III. *Rhytidoponera araneoides*, however, has expanded to the Philippines and Solomon Islands and is classified as Stage I (secondary). *a* represents the bulk of the Papuan species, which are confined to the New Guinea mainland; *b*, *R. nexa*; *c*, *R. araneoides*; *d*, six endemic New Caledonian species, whose closest affinities are with Australian stocks.

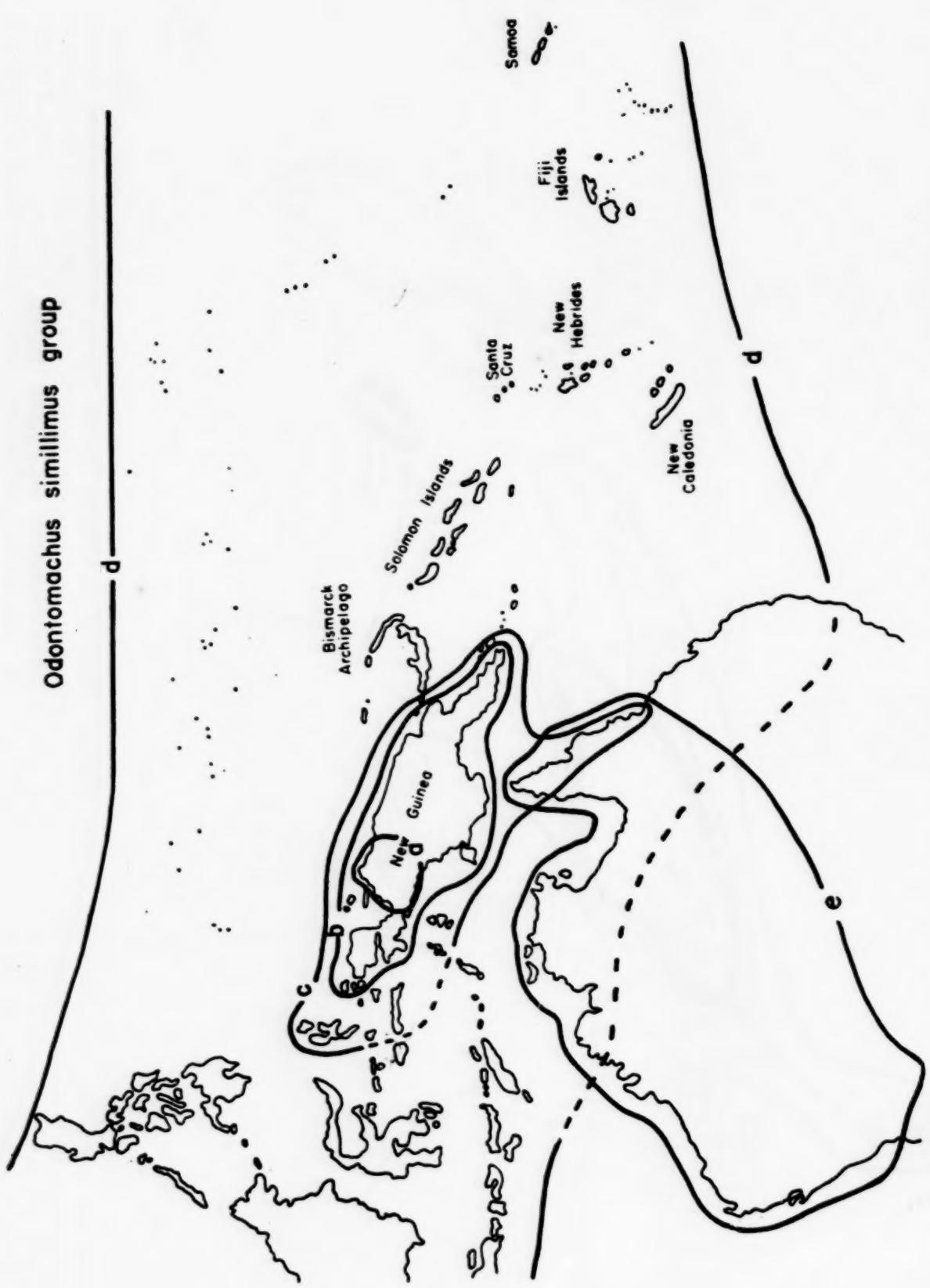
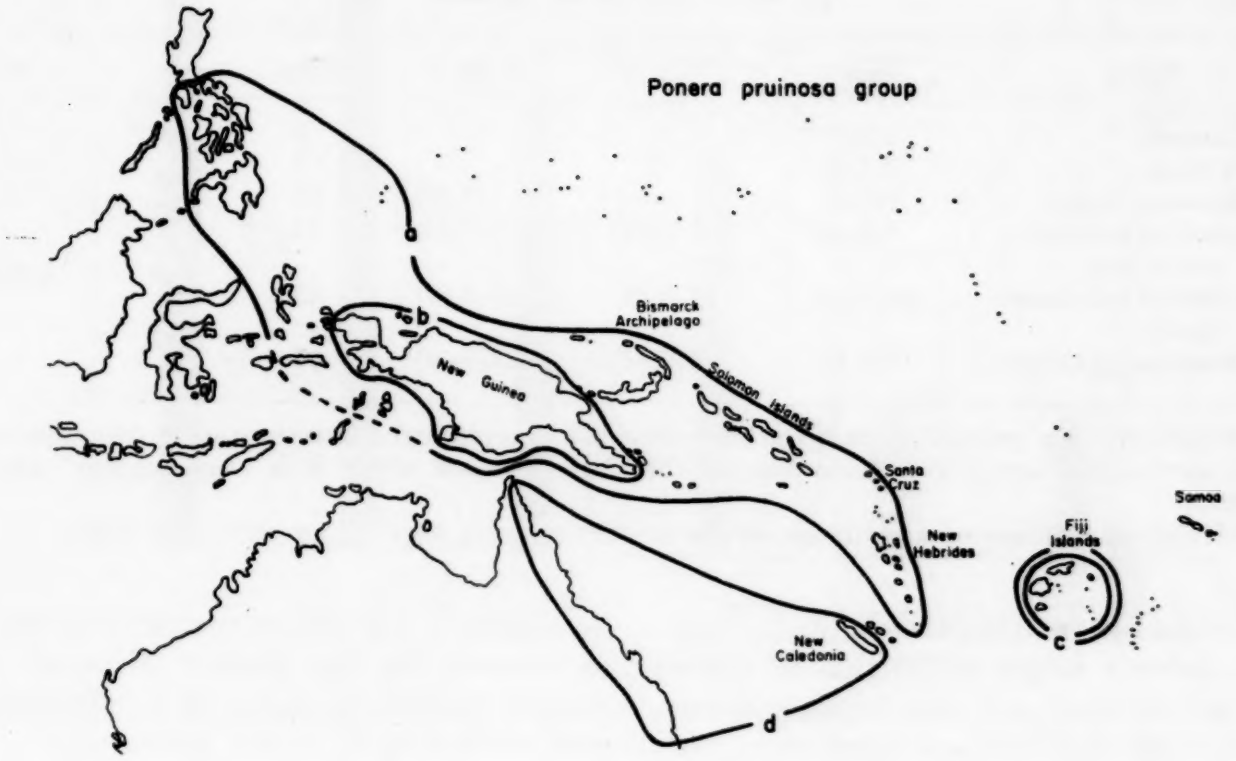


FIG. 7. Maximum known distributions of species of the *Odontomachus simillimus* group. Classified variously in Stage I (secondary) or Stage III, depending on their present ranges. *a*, *O. aeneus*; *b*, *O. aciculatus*; *c*, *O. cephalotes*; *d*, *O. cimillimus*; *e*, *O. ruficeps*. *O. simillimus* (= *O. haematodus auct.*) ranges over most of the Pacific and has probably been transported through a large part of this area by man.



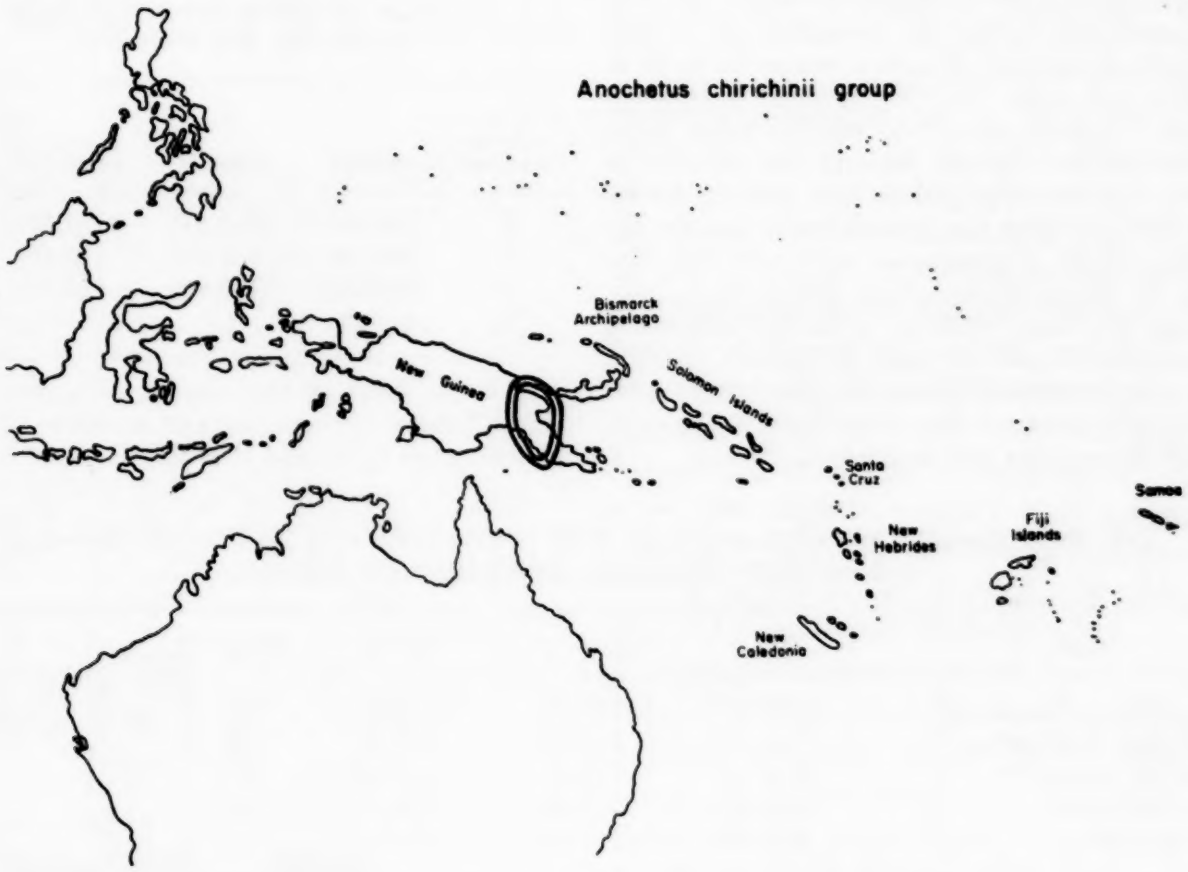


FIG. 8. Maximum known distributions of species in late Stage III. Upper: *a, Ponera pruinosa; b, P. sabronae; c, P. monticola* and *P. vitiensis; d, P. elliptica.* Lower: *Anochetus chirichinii* and *A. vesperus.* All of these species are classified in Stage III except *Ponera pruinosa,* which has expanded to outer Melanesia, the Philippines, and Palau. *P. elliptica* is not considered in the present analysis.

TABLE 2. *Partition by evolutionary stage of the Ponerine fauna in six major New Guinea habitats*

Habitat	Stage I	Stage II	Stage III	Total	χ^2	P*
a. Littoral	1(*1.0*)**	—	—	1	0	
b. Savanna	2(*1.0*)	—	—	2	0.41	
c. Monsoon forest	8(*0.8*)	—	2(*0.20*)	10	0.81	
d. Lowland rain forest (marginal)	9(*0.69*)	1(*0.08*)	3(*0.23*)	13	8.08	<0.02
e. Lowland rain forest (interior)	12(*0.27*)	13(*0.29*)	20(*0.44*)	45	0.17	
f. Montane rain forest	15(*0.24*)	20(*0.32*)	27(*0.44*)	62		

* P indicates the probability that the evolutionary and ecological characteristics in two adjacent rows are independent. It is given here in the single instance where it is "significantly" small (<0.05).

** Italicized numbers in parentheses are the proportions with respect to evolutionary stage.

to conduct normal nuptial flights.[3] Stage-I includes a larger proportion of species nesting in open soil and beneath stones, as opposed to rotten wood and trees, than do Stages II and III, so that it is not easy to account for the greater dispersal of Stage-I species by means of a differential rate of "rafting."

[3] The existence of a normal nuptial flight is no guaranteee of a relatively high potential dispersal rate, since the organization of the flight itself may be of such a nature as to limit greatly this rate. In the Nearctic myrmicine species *Pheidole sitarches,* mating takes place on the ground directly beneath the swarms of males, and the queen does not attempt to fly afterward, so that the species range can be advanced within a generation only over the distance by which the males are able to form their swarms (Wilson, 1958c). If similar behavioral phenomena occur in Indo-Australian species, they can presumably normally cross water gaps only if the gaps are less than the distance over which the swarms can be formed.

TABLE 3. *Partition by evolutionary stage of species occurring in various numbers of major habitats in New Guinea* *

Number of habitats occupied	Stage I	Stage II	Stage III	Total
1	5(*0.09*)	19(*0.33*)	33(*0.58*)	57
2	6(*0.30*)	6(*0.30*)	8(*0.40*)	20
3	2(*0.40*)	2(*0.40*)	1(*0.20*)	5
4	6(*1.0*)	—	—	6

* On the basis of two classes, occupants of a single habitat vs. occupants of multiple (2, 3, 4) classes, $\chi^2 = 16.62$ and P = 0.001.

TABLE 4. *Partition by evolutionary stage of the species comprising the Ponerine faunas of various of the Melanesian Archipelagos and Samoa*

Place	Stage I	Stage II	Stage III	Total	χ^2	P
New Guinea Mainland*	24(*0.22*)	35(*0.31*)	53(*0.47*)	112	10.52	0.005
Bismarck Archipelago**	10(*0.56*)	1(*0.06*)	7(*0.38*)	18	1.67	
Solomon Islands**	18(*0.49*)	7(*0.19*)	12(*0.32*)	37	3.83	
New Hebrides**	9(*0.82*)	1(*0.09*)	1(*0.09*)	11	11.10	0.001–0.002
Fiji Islands*	3(*0.18*)	7(*0.41*)	7(*0.41*)	17		
Samoa**	5(*0.83*)	1(*0.17*)	—	6	8.69	0.01–0.02

* The New Guinea and Fijian faunas do not differ significantly from each other in proportionment by evolutionary stage; χ^2 is only 0.64.

** The faunas of central Melanesia (Bismarcks, Solomons, New Hebrides) and Samoa do not differ significantly from each other in proportionment by evolutionary stage; χ^2 between New Hebrides and Samoa is 0.87.

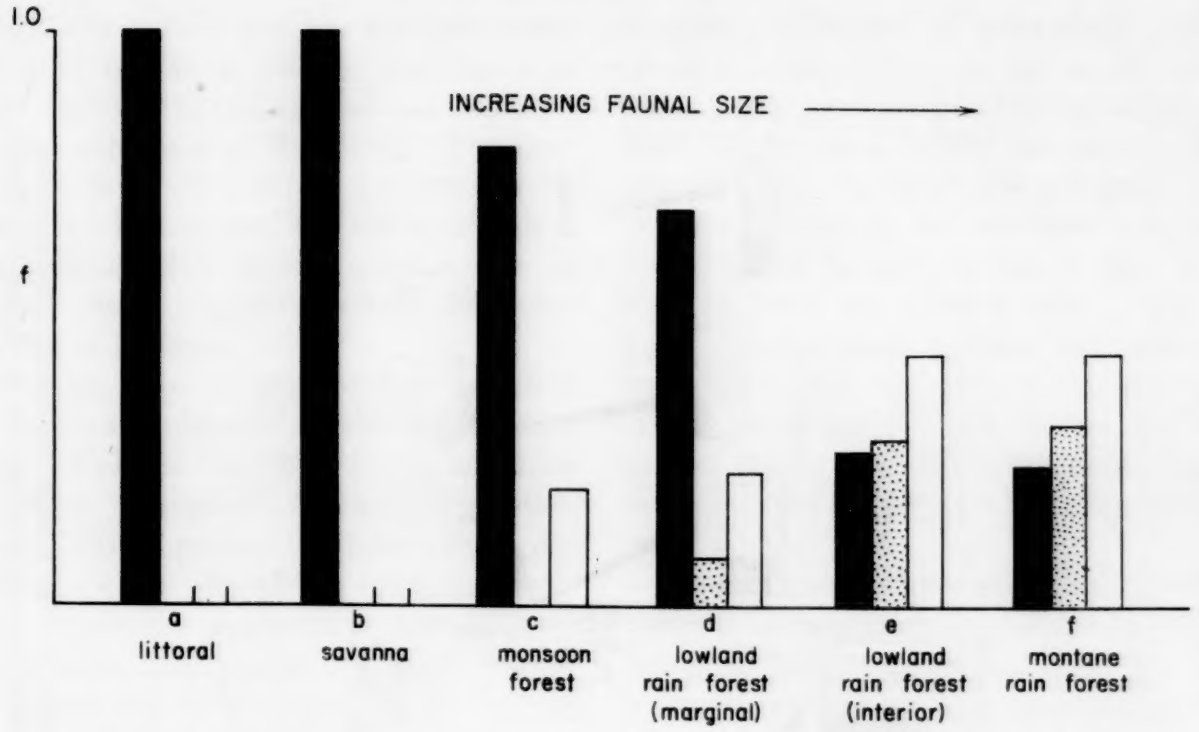


FIG. 9. Partition by evolutionary stage of the ponerine faunas of various major habitats in New Guinea. Black, Stage-I species; stippled, Stage II; blank, Stage III. See table 2.

Is it possible that faunal movement occurs through marginal habitats simply because these habitats are more often nearer the coast? The present pattern of vegetation on New Guinea does not lend strong support to such a view. It is true that mountains, and montane rain forest, containing the largest number of ponerine species, are predominantly inland. But it is also generally true that where rainfall is sufficient to maintain lowland rain forest, this forest extends right to the coast, and where rainfall is not sufficient, grassland and monsoon forest extend far inland. It does not seem possible to invoke the slightly greater geographic accessibility of marginal habitats as the deciding factor in the marked preference of Stage-I species for these habitats.

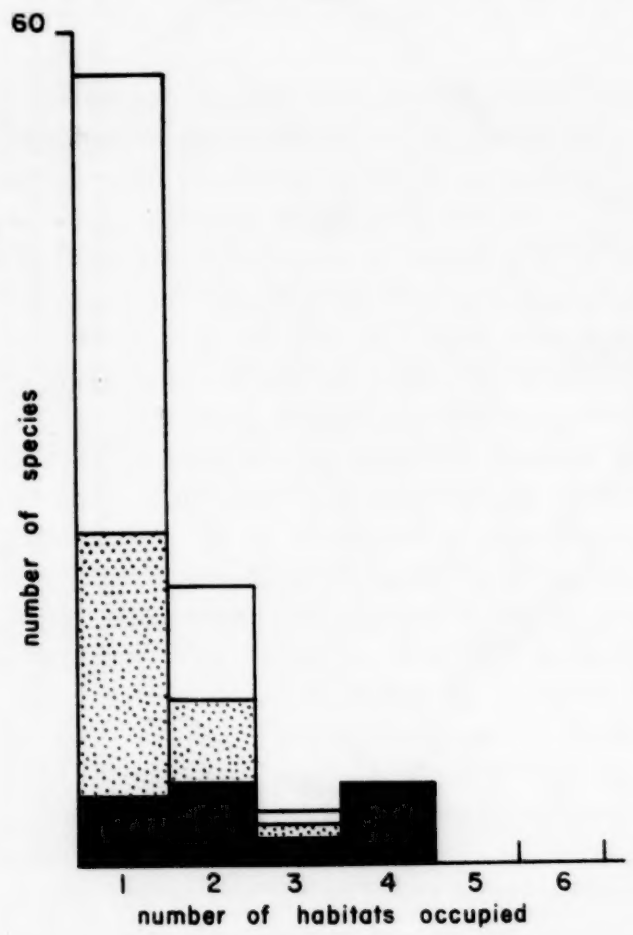


FIG. 10. Relationship of evolutionary stage and number of habitats occupied in the New Guinea fauna. Conventions as in figure 8. See table 3.

Perhaps a stronger possibility to be considered is that the fauna of the marginal habitats is more prone to dispersal by wind and water, since it is more open to the action of wind currents and tends to be distributed differentially near waterways, e.g., "open aspect" rain forest predominates along streams in forested areas. There is at the present time insufficient evidence to test the validity of this supposition. If anything, two characteristics of Stage-I species, the common occurrence

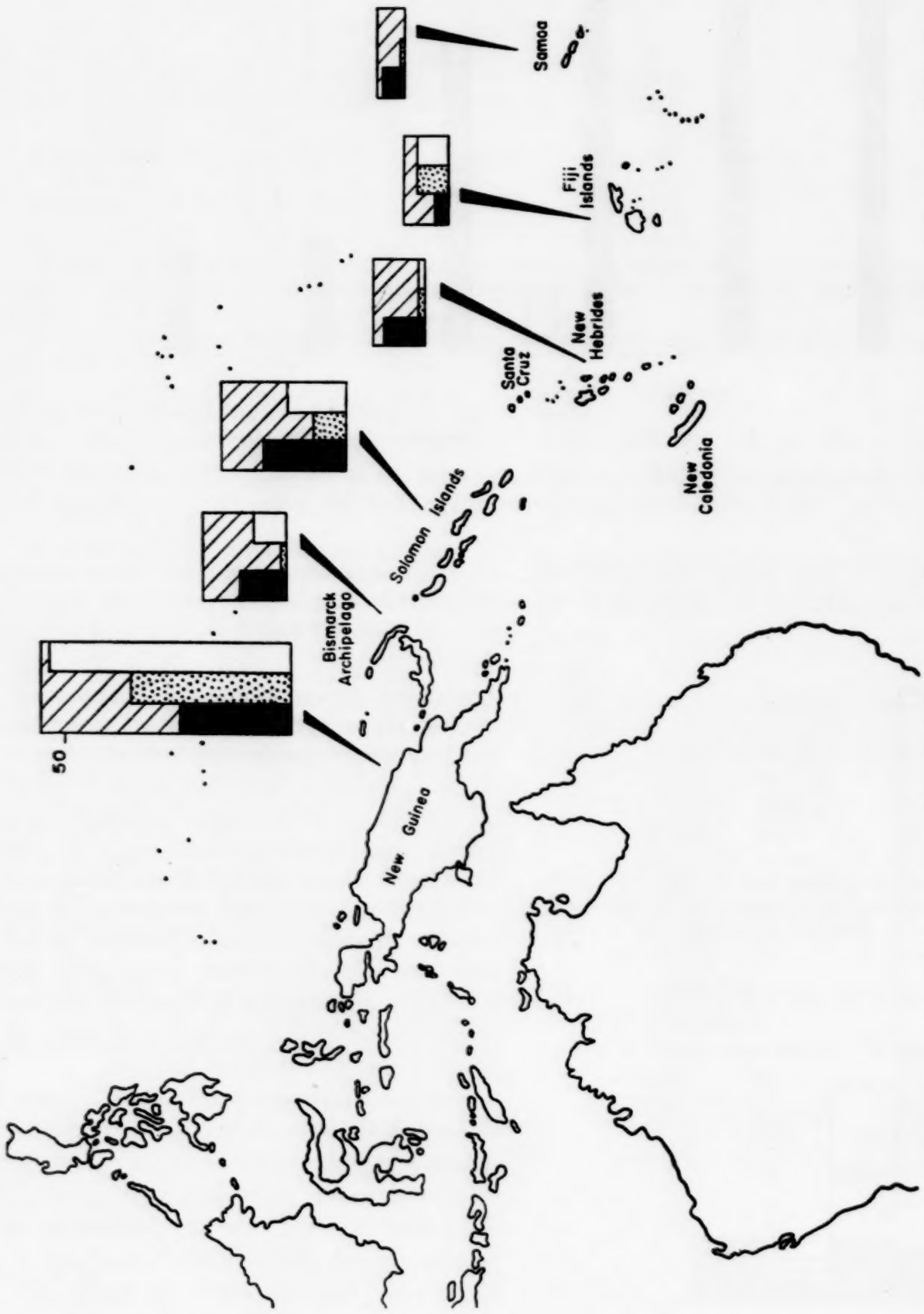


FIG. 11. Number of species groups and partition of species by evolutionary stage in the faunas of various Melanesian archipelagos. Hatched column represents the number of species groups; otherwise conventions are as in figure 9. The size of the Bismarck fauna as given is probably not indicative of its true relative size, since it is the most poorly explored; it is probably larger than that of the Solomons. See table 4.

of flightless queens and the common preference of soil as a nesting site, seem to weigh against regarding such an explanation too seriously at this time. Furthermore, there is some direct evidence, to be considered below, that the resident Stage-II and Stage-III species play a role in keeping Stage-I species out of the inner rain forest habitat.

It might also be argued that parts of the Celebes-Moluccan corridor, including some of Celebes itself and Buru, are drier and more "marginal" in their vegetation cover, hence posing a filter that has favored species adapted to marginal habitats. Again the evidence is not adequate to evaluate this possibility, and it may not be soluble until ecological studies are carried out in the Celebes and Moluccas. At least it is known that vast areas of these islands, including the entire island surfaces in some cases, are covered with dense rain forests, thus seeming to provide excellent stepping-stones that would allow a true rain forest fauna to cross over to New Guinea.

If the provisional interpretation adopted here is true, that different dispersal mechanisms and purely geographic phenomena are not sufficient to account for the marginal-habitat effect, one is inclined to turn to features of the biotic environment within Melanesia for a more complete explanation. There is likely significance in the fact that marginal habitats, containing as they do the smallest number of species, offer the least diversified biotic resistance. It is in fact the hypothesis advanced here that Stage-I species, by penetrating marginal habitats initially, are "flanking" the competition of the resident fauna rather than undertaking a "frontal assault" on it. The ant fauna of the inner rain forest does indeed present the appearance of a formidable closed "association." The species are very numerous (over 160 at the lower Busu River alone) and highly specialized to fill, in the aggregate, almost every imaginable niche available to ants. Moreover, the absolute composition of local faunas, as well as the relative abundance of individual species, changes over distances as short as 12 kilometers, producing a "kaleidoscope effect" in the total faunal structure (Wilson, 1958d). In short, the potential competition offered by the resident inner rain forest fauna is very great at any given locality, and its overall effect may be greatly heightened by the spatially and temporally shifting nature of the total faunal structure, which would serve to thwart the extension of any local adaptation achieved by the first invading populations.

Now the question must be raised, if Stage-I species normally enter by way of marginal habitats, why has there not been a faunal buildup in these habitats, eventually closing them to further invasion? There is abundant evidence to indicate that species do not linger long in the marginal habitats. By the time they have differentiated and achieved Stage-II status, they usually have succeeded in penetrating the deep rain forest habitat.[4] Moreover, there is some indication that Stage-I species enter deep rain forest wherever competitive forms are relatively scarce. Thus *Rhytidoponera araneoides* is restricted to marginal habitats in New Guinea, but is apparently abundant in deep rain forest in the Solomon Islands, where the ponerine and myrmicine fauna is significantly smaller. On the Huon Peninsula of New Guinea, which has one of the largest and most diversified ponerine-myrmicine faunas of any comparable area in the world, *Odontomachus simillimus* (= *haematodus auct.*) is limited to marginal habitats. In the vicinity of the Brown River, Papua, with a local fauna only slightly larger than half that of local

[4] For the Ponerinae the most favored type of habitat in the New Guinea lowlands is what I have described elsewhere as "medium aspect" rain forest (Wilson, 1958d). Within this habitat most Stage-II and Stage-III ponerine species nest on the ground in rotting logs at the "zorapteran" and "passalid" stages of decomposition and prey on small arthropods. Microhabitat and nest-site choice is more variable in the mountain rain forests.

faunas on the Huon Peninsula, *O. simillimus* is moderately abundant in primary rain forest as well as marginal habitats. On Espiritu Santo, New Hebrides, which has a depauperate fauna, *O. simillimus* is one of the dominant ants on the floor of primary rain forest.

At this point the possibility should be considered that the invasion of Melanesia by way of the marginal habitats is a recent, unique event and not a continuing historical process, as supposed. This would be true if the coming of man, or else extensive drying of parts of New Guinea during the Pleistocene independent of man, had been necessary to open marginal habitats sufficiently for the Stage-I species to invade. In the author's opinion it is highly unlikely that such a circumstance existed. Even if at various geologic periods there were no savanna or monsoon forest, there would always be extensive rain forest border, and within the rain forest abundant patches of open-aspect, second-growth vegetation resulting from the fall of large trees, stream erosion, and landslides. Anyone who has worked in primary tropical forest is familiar with the extensiveness of these phenomena. Native cultivation on New Guinea has undoubtedly greatly increased the extent of marginal habitats, and along with it the size of Stage-I populations, but if its effects were to be obliterated overnight, there would still be sufficient marginal areas to support large Stage-I populations. The same is probably true if all savannas and monsoon forests present in New Guinea today were to be replaced by continuous rain forest; there would remain abundant patches of open-aspect vegetation within the forest.

For invading (Stage-I) species, entering New Guinea by the marginal habitat route, there is a strong selective pressure to penetrate the inner rain forest (see fig. 12). Apparently they must achieve this adaptive shift in a relatively short time or face extinction, either through unfavorable environmental changes or under the pressure of newly invading competitor species. In evidence is the fact that there are relatively few old endemic species in the marginal habitats and even fewer that appear to be confined to them. This situation poses something of a paradox,

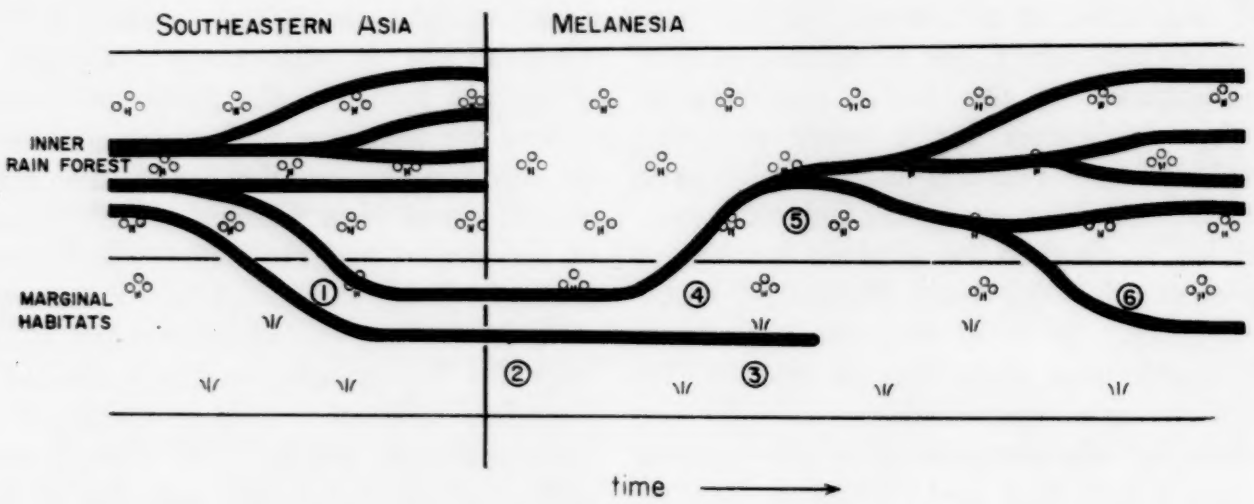


FIG. 12. Schematic representation of the hypothesized evolution of ponerine species groups in Melanesia, in this case tracing the history of groups derived ultimately from Asia. (1) Species or infraspecific populations adapt to marginal habitats in southeastern Asia, then cross the water gap to New Guinea and colonize marginal habitats there (2). In time these Stage-I species either become extinct (3) or invade the inner rain forest of Melanesia (4). Having successfully adapted to the inner rain forest, they diverge to species level (5), thus entering Stage II. As diversification continues in Melanesia, the source fauna in Asia may be contracting, so that in time the group as a whole becomes Melanesian-centered and its Melanesian species are classified as Stage III. A few of the New Guinea species may readapt to the marginal habitats (6) and expand back out of New Guinea, thus entering Stage I secondarily.

for it is true that the marginal habitats support (at least at present) large populations of ants. For the period of their tenure in the marginal habitats, the Stage-I species appear to be relatively abundant. What, then, causes their frequent extinction and the resultant selective pressure to shift into the deep rain forest habitat? Two causal phenomena can be hypothesized: (1) the environment of the marginal habitats, both physical and biotic, is more variable and capricious; (2) historically the marginal habitats have been limited in size and diversity of available niches, so that resident populations have tended to remain genetically relatively homogeneous and hence more vulnerable to the deleterious effects of rapid environmental change. (It should be noted that while the marginal habitats have fewer niches, it is thought that these niches tend to remain more open than is the case in rain forest, because of greater fluctuations in population size of resident species.)

Thus it is conceivable that the marginal habitats provide an opening for invading species while at the same time serving as an evolutionary trap for those that remain in residence. The islands of outer Melanesia may function in a similar fashion. As shown here, all of the archipelagos, with the single exception of the Fiji Islands, contain a higher proportion of Stage-I species than New Guinea. This youthful character of the fauna of central Melanesia may be due to the fact that these islands are geologically younger or less stable. But it may be also true that their smaller size and less diversified biota cause them to function also as evolutionary traps, "weakening" resident species and rendering them more susceptible to competition from subsequently invading species. The idea that oceanic islands do in fact have such a general effect on the evolution of endemic faunas has already been developed at some length by Mayr (1942, 1954) and Darlington (1957) and does not need further expression here.

Dobzhansky (1955) has recently summarized the population geneticist's view of the process of adaptation to diversified environments in the following rule: "Granted that genetic variability is an instrumentality whereby Mendelian populations master environmental diversity, one may expect that, other things being equal, populations which control a greater variety of ecologic niches will be more variable than those having a more limited hold on the environment." In a penetrating analysis extending over the past ten years, Dobzhansky and his colleagues have gone far to document just a phenomenon in the *Drosophila willistoni* group (da Cunha, Burla, and Dobzhansky, 1950; da Cunha and Dobzhansky, 1954; Birch and Battaglia, 1957; Dobzhansky, 1957; Townsend, 1958). Although the evidence is of a different and more limited kind, one is tempted to extend Dobzhansky's rule to include consideration of the evolution of the Melanesian ponerines. At first glance there appears to be a basic contradiction. If Stage-I species tend to be genetically impoverished, as suggested in the present paper, how is it that they occur in a greater array of major habitats than do old resident species? Would not greater ecological amplitude of this nature presuppose greater genetic diversification? The answer suggested by our much more extensive knowledge of *Drosophila willistoni* is that occurrence of a species or a population of a species in a wide range of major habitats (as defined here) does not of itself imply genetic diversity. If the habitats are marginal, or the distribution peripheral, the species (or population) may be relatively homogeneous. Of much greater importance are length of residence within the part of the range under consideration and the microecological diversification within the major habitats occupied. In the case of *Drosophila willistoni* it is the tropical forests and adjacent savannas of central Brazil that contain the largest number of inversion types and the highest percentage of in-

version heterozygotes. The Brazilian forests appear to be the center of the range of the species and also that part of the range to which resident populations are maximally adapted. In the case of the Melanesian ponerines, most of the Stage-I species are derived from Oriental stocks that are probably primarily rain-forest dwellers. In the case of these that have reached Stage-I status by expanding out of New Guinea by way of the marginal habitats, it is known with certainty that they belong to groups that are otherwise adapted to the inner rain forest. In making an adaptive shift to marginal habitats, the pioneer populations of these stocks are entering a peripheral, generally less favorable area, and it is probable that their genetic variability is correspondingly diminished. Furthermore, as Mayr (1954) has duly emphasized in his elaboration of the "founder principle" in speciation, pioneer populations such as those crossing water gaps in Melanesia are certain to have diminished variability, and the consequently narrowed "genetic environment" may have an accelerating effect on subsequent evolution.

With the foregoing theoretical background in mind, it is now possible to construct a model of the sequence of genetic events that have occurred in the colonization of Melanesia by the ponerine ants. Five steps can be conceived.

1. Prior to its invasion of New Guinea, the species occupies a central range, or permanent breeding area, probably composed mostly of deep rain forest, and on occasion marginal areas as well, in sum making up what Brown (1957) has referred to as the "maximum range." Brown has suggested the following events as being typical for animal populations: "Within the maximum range, the populations of the species normally undergo successive expansions into the less favorable areas, alternating with contractions into more favorable refuges. The expansions and contractions are the sequelae of inevitable density fluctuations affecting all or part of the species at one time."

2. Evolutionary changes accompany the continuing process of expansion and contraction. Populations occupying marginal areas will tend to adapt to the differing habitats found there.

3. Populations that are adapted to marginal habitats are now candidates for the colonization of New Guinea. Queens and colony fragments from both rain-forest and marginal-habitat populations probably disseminate from time to time across the water gaps to New Guinea, but generally only those adapted to marginal habitats are able to establish pioneer populations. The basis of this selection is the more open nature of the resident endemic marginal-habitat populations on New Guinea, containing fewer species and offering overall less competition to invading forms.

4. Having become established in New Guinea, the pioneer populations tend to remain genetically homogeneous because of the "niche-poor" condition of the marginal habitats. Their future is rendered further hazardous by the more intense fluctuations that occur in the marginal environments. Hence there is a strong selective pressure on these populations to penetrate the inner rain forest habitat.

5. If penetration of the inner rain forest is successful, new evolutionary opportunities are realized, and the populations tend to diversify and speciate. In time Group-II status is reached. With the further passage of time, however, the phyletic line will ultimately tend to diminish, as new, better adapted stocks penetrate the inner rain forest from outside. Occasionally, a species will break out of the inner rain forest habitat secondarily and expand out of New Guinea in the same fashion that the ancestral species entered, by way of the marginal habitat route. Such species, however, rarely if ever are able to push back beyond Australia or the Philippines.

Summary

1. The zoogeography of the Melanesian ponerine fauna is preliminarily analyzed. Most of the fauna has apparently been

derived ultimately from Oriental stocks entering by way of New Guinea; some invading species are able to spread beyond this island to Queensland and outer Melanesia. A smaller part of the fauna has been derived from old Australian stocks that have entered by way of New Guinea or New Caledonia. Faunal flow from New Guinea through outer Melanesia has been unidirectional, with an ever diminishing number of species groups found outward from the Bismarcks to the Fiji Islands. New Caledonia draws almost all of its fauna from eastern Australia and has engaged in very little direct faunal exchange with the remainder of Melanesia.

2. A cyclical pattern of expansion, diversification, and contraction is hypothesized to account for later evolutionary events following initial dispersal. Following invasion of Melanesia (Stage I, *primary*), the pioneer populations may then diverge to species level (Stage II) and further diversify. Eventually the source populations outside Melanesia tend to contract, leaving the species group as a whole peripheral and Melanesian-centered (Stage III). Endemic Melanesian species occasionally enter upon a secondary phase of expansion (Stage I, *secondary*) but are rarely if ever able to push beyond Australia or the Philippines.

3. Stage-I species are characterized on New Guinea by their greater concentration in "marginal" habitats, including open lowland forest, savanna, and littoral. The central habitats, including denser lowland forest and montane forest, contain significantly larger faunas as well as a higher percentage of Stage-II and Stage-III species. Stage-I species are also characterized by their individual occurrence in a greater range of major habitats. Finally, these species make up a significantly higher proportion of the faunas of the archipelagos of central Melanesia, including the Bismarck Archipelago, the Solomon Islands, and the New Hebrides.

4. On the basis of these data it is suggested that ponerine species normally invade New Guinea by way of the marginal habitats. Evolutionary opportunity is nevertheless limited in the marginal habitats, and there is a strong selective pressure favoring re-entry into the inner rain forest habitats. In general, Stages II and III, leading to the origin of the great bulk of the Melanesian fauna and its most distinctive endemic elements, are played out only in the inner rain forest.

Acknowledgments

The author wishes to acknowledge his gratitude to W. L. Brown, P. J. Darlington, T. Dobzhansky, A. E. Emerson, C. Lindroth, E. Mayr, and E. C. Zimmerman for reading the manuscript and offering many helpful criticisms and suggestions.

Literature Cited

Birch, L. C., and B. Battaglia. 1957. Selection in *Drosophila willistoni* in relation to food. Evolution, **11**: 94–105.

Brown, W. L. 1957. Centrifugal speciation. Quart. Rev. Biol., **32**: 247–277.

——. 1958. Contributions toward a reclassification of the Formicidae. II. Tribe Ectatommini (Hymenoptera). Bull. Mus. Comp. Zool. Harvard, **118**: 175–362.

da Cunha, A. B., H. Burla, and Th. Dobzhansky. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution, **4**: 212–235.

da Cunha, A. B., and Th. Dobzhansky. 1954. A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. Evolution, **8**: 119–134.

Darlington, P. J. 1957. Zoogeography. Wiley.

Dobzhansky, Th. 1955. A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symp. Quant. Biol., **20**: 1–15.

——. 1957. Genetics of natural populations XXVI: chromosomal variability in island and continental populations of *Drosophila willistoni* from Central America and the West Indies. Evolution, **11**: 280–293.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press.

——. 1954. Change of genetic environment and evolution. In Evolution as a process. Allen and Unwin.

Richards, P. W. 1952. The tropical rain forest. Cambridge University Press.

TOWNSEND, J. I. 1958. Chromosomal polymorphism in Caribbean island populations of *Drosophila willistoni*. Proc. Nat. Acad. Sci., **44**: 38–42.

WILSON, E. O. 1957. The *tenuis* and *selenophora* groups of the ant genus *Ponera* (Hymenoptera: Formicidae). Bull. Mus. Comp. Zool. Harvard, **116**: 355–386.

——. 1958a. Studies on the ant fauna of Melanesia. I. The tribe Leptogenyini. II. The tribes Amblyoponini and Platythyreini. Bull. Mus. Comp. Zool. Harvard, **118**: 101–153.

——. 1958b. Studies on the ant fauna of Melanesia. III. *Rhytidoponera* in western Melanesia and the Moluccas. IV. The tribe Ponerini. Bull. Mus. Comp. Zool. Harvard, **118**: 303–371.

——. 1958c. Organization of a nuptial flight of the ant *Pheidole sitarches* Wheeler. Psyche, **64**: 46–50.

——. 1958d. Patchy distributions of ant species in New Guinea rain forests. Psyche (In press.)

NOTES AND COMMENTS

VARIABILITY AND ENVIRONMENT

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In a brief survey of recent Russian work on the composition of ecotypic and varietal populations, Sinskaya (1958) has commented on the enhancement of variability which may result when samples of a phenotypically uniform population are transferred to localities differing widely from that to which they are habituated. Clausen and Hiesey (1958) in the same journal discuss similar problems under the title *Phenotypic expression of genotypes in contrasting environments.* Notwithstanding differences in expression and terminology, the two articles are concerned to make the same point: that outbreeding populations carry a reserve of genetical variability which may not be expressed phenotypically in their normal environments, but which may be exposed in other environments and then become open to selection. That this is often true is not to be doubted. Nowhere is the effect more striking than in the responses of plants to photoperiodic and temperature regimes, where a transfer to a foreign environment may result in a spectacular increase in the variation between individuals in rate of development towards flowering. Excellent examples have been described in *Bouteloua* (Olmsted, 1944), *Deschampsia* (Lawrence, 1945), *Sorghum* (Quinby and Karper, 1945), and *Lolium* (Cooper, 1954). Whilst in these cases the interpretation is in little doubt, it seems desirable to point out that not always can the enhancement of phenotypic variability in a foreign environment be attributed to exposure of latent genetical variability. One of the examples of Sinskaya concerning the behavior of the labiate *Perilla ocymoides* is open to a very different explanation. *Perilla* is highly sensitive photoperiodically, flowering early in short days, and very late in continuous illumination. The variety described by Sinskaya showed a high degree of uniformity when grown throughout life in either a short-day or a long-day regime. However, when grown for two or three weeks from germination in short days and then transferred to long days, the same variety showed ". . . a fabulous disintegration into morphobiological types. . . . Certain features of these plants are beyond the limits of our present-day knowledge of the variability of the given species and even family (for example, the bow-shaped nervature of the leaf instead of the reticulate one)." It seems probable that this particular case of enhanced phenotypic variability is due not to an exposure of latent genetic variation, but to the severe dislocation of developmental regulation induced in each individual suffering the abrupt and highly unnatural change in photoperiod. The greater variation amongst individuals is one expression of a general increase of morphogenetic instability arising from the break-down in developmental regulation or "buffering." Far from necessarily indicating latent genetic variation, it may even point to an abnormally high level of homozygosity, if developmental homeostasis (or better, "homeorhesis," Waddington, 1957) is indeed promoted by heterozygosity (Lerner, 1954). Although Sinskaya's use of the word "disintegration" to cover all examples of enhanced phenotypic variability is confusing, it seems that a distinction of this kind may be intended when she writes, "Such a mode of disintegrating populations (beyond the limits of their own norms of reaction and those of their components) should be distinguished from the state of falling into eco-elements which are the initial points or nodes of natural selection activity."

Yet a further distinction can be made, between the enhanced variability apparent in populations during periods of very rapid developmental change in environments within the normal range, and that arising in environments radically different from the normal. An example of the former, in a sample of male plants of *Cannabis sativa* (hemp), is given in table 1. Like *Perilla,* hemp is photoperiodically sensitive, flowering

TABLE 1. *Means and coefficients of variation for marginal serration of central lobes of leaves of male plants of hemp.* Node 6, leaves differentiated under two daily light periods of 8 hrs; Node 10, leaves differentiated after change to one daily light period of 8 hrs.

	Node 6	Node 10
Mean serration number	13.41±0.171	8.82±0.374
Coefficient of variation*	5.34±0.904	17.74±3.089

* Calculated with Haldane's correction.

TABLE 2. *Within-plant variances of perianth and androecium of male plants of hemp.* Single photoperiod of 8 hrs per 24 hrs; "day" temperature 22° C. Six plants per series; samples of 25–70 flowers per plant.

Night temperature	Tepal number	Stamen number	Degrees of freedom
22° C	0.0185	0.0437	263
10° C	0.3290	0.6601	245
	$p \ll .01$	$p \ll .01$	

when exposed to short days. The plants in this experiment were of a fiber strain closely inbred for eight generations and rigorously selected for uniformity in photoperiodic response and absence of developmental anomalies. They were grown throughout in a fully controlled environment, with temperature held at 22° C, relative humidity at 75%, and a standard level of mineral nutrition. Light was supplied at 1100 f. c. from a warm-white fluorescent source; from germination to an age of 20 days the plants received two daily light periods of 8 hrs spaced by 4-hr periods of darkness, and from this age onwards, one 8-hr light period daily. As a measure of variability, the numbers of serrations on one margin of the central lobe of the leaves may be taken; this is a conveniently recorded index of leaf venation (Heslop-Harrison and Heslop-Harrison, 1958). The last leaf differentiated in the original photoperiod was at node 6, and the extreme uniformity of the culture is evident from the low value of the coefficient of variation for its marginal serration. For leaves formed four nodes later, after the abrupt change in photoperiod, the coefficient of variation rose by a factor of rather more than three. Uniformity was reached again in the neighborhood of the inflorescence, where the leaves were reduced to small, entire bracts. The interpretation of the effect appears to be simply that when developmental changes are progressing slowly at the stem apex, minor fluctuations in the times at which leaf primordia are initiated have little phenotypic effect, but when rapid changes in developmental rate are initiated—in this instance by exposure to a photoperiodic regime promoting flowering—the small variations in rates of leaf production between individuals are likely to result in considerable differences in leaf pattern.

A striking case of the effects of severe environmental stress upon developmental regulation, also in male plants of hemp, is recorded in table 2. The plants in this experiment received the same treatment as in that just described, but at the time when the photoperiod was changed, one group was transferred to a temperature regime of 22° C during the light period and 10° C during the dark, whilst a control group remained throughout at 22° C. The group receiving cool nights was considerably retarded in development, and when flowering began the amount of meristic variation in corolla and androecium was substantially greater than normal. The comparison of within-plant variances for tepal and stamen number given in table 2 indicates clearly that the effect is upon the developmental regulation of the individuals. Undoubtedly this particular combination of temperatures and photoperiods represents a somewhat abnormal environment for hemp; yet the plants were able to maintain themselves and reproduce successfully, notwithstanding the number of morphogenetic abnormalities. Floral structures are normally the most stable of the plant, and yet it is clear that towards the limit of ecological tolerance conditions may be encountered in which phenotypic variability is enormously enhanced.

There are obvious implications for plant population studies and transplant experiments in these results. Investigators are normally awake to the fact that the phenotypic variability observed in population samples is the product both of genotypic variation and plastic modification of individuals resulting from minor differences in their environments; but not all will be prepared for the possibility that in different, wholly uniform environments non-genetic variation may itself vary in amplitude due to differences in the efficiency of developmental regulation. This additional dimension of variation introduces a confusing factor into the interpretation of transplant experiments, and its existence reinforces the recommendations of Hiesey and of Went (1957) that controlled-environment equipment should be more extensively used in the study and analysis of ecological races.

LITERATURE CITED

CLAUSEN, J., AND HIESEY, W. M. 1958. Phenotypic expression of genotypes in contrasting environments. Scottish Plant Breeding Stn., Report for 1958: 41–51.

COOPER, J. P. 1954. Studies on growth and development in *Lolium*. IV. Genetic control of heading responses in local populations. J. Ecol., **42**: 521–556.

HESLOP-HARRISON, J., AND HESLOP-HARRISON, Y. 1958. Studies on flowering-plant growth and organogenesis. III. Leaf shape changes associated with flowering and sex differentiation in *Cannabis sativa*. Proc. Roy. Irish Acad. B, **59**: 160–195.

HIESEY, W. M. 1957. Climatic response of grasses. Chap. 11, Experimental control of

plant growth (Ed. F. W. Went). Chronica Botanica, Waltham, Mass.

LAWRENCE, W. E. 1945. Some ecotypic relations of *Deschampsia caespitosa*. Amer. J. Bot., **32**: 298–314.

LERNER, I. M. 1954. Genetic Homeostasis. Oliver and Boyd Ltd., Edinburgh.

OLMSTED, C. E. 1944. Growth and development in range grasses. IV. Photoperiodic responses in twelve geographic strains of side-oats grama. Bot. Gaz., **106**: 46–74.

QUINBY, J. R., AND KARPER, R. E. 1945. The inheritance of three genes that influence time of floral initiation and maturity date in milo. J. Amer. Soc. Agron., **37**: 916–936.

SINSKAYA, E. N. 1958. Investigations on the composition of ecotypical and varietal populations. A brief survey of some of our works published in Russian. Scottish Plant Breeding Stn., Report for 1958: 31–40.

WADDINGTON, C. H. 1957. The strategy of the genes. George Allen and Unwin Ltd., London.

WENT, F. W. 1957. Experimental Control of plant growth. Chronica Botanica, Waltham, Mass.

THE ANTENNAE OF DROSOPHILA FEMALES

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Ernst Mayr (1950) investigated the role of the antennae in the mating behavior of *Drosophila* females by removing the antennae of *D. pseudoobscura* females in *D. pseudoobscura* female × *D. persimilis* male crosses. Despite "a general lowering of vitality as a result of the operation," the removal sufficed to significantly reduce the sexual isolation between the two species. Where heterogamic mating took place 2.5% of the time under normal conditions, it was increased to 26.8% with the elimination of the female antennae as organs of discrimination.

I have applied this same technique to crosses involving *D. insularis* × the other sibling species of the *willistoni* species group—*tropicalis, willistoni, equinoxialis,* and *paulistorum,* in multiple choice experiments. (See Dobzhansky et al., 1957.) Of the 936 females dissected, only thirty had been inseminated. (Controls showed 83.3 to 100% conspecific insemination.) While this positive number is too small for further analysis, it should be pointed out that five of the thirty were indeed heterogamic matings.

This is reminiscent of Spieth's (1952) work with *D. virilis* wherein removal of the fore tarsi in crosses between two strains altered the behavior of one of the strains, while the other failed to receive stimuli, and was, therefore, not moved to action. Apparently, removal of the antennae of the willistoni group females causes the almost complete cessation of mating.

To check on my technique I repeated Mayr's experiment with *pseudoobscura* females × *persimilis* males. The dissection of 280 ♀♀ proved his data to be accurate. Certain vials gave as high as 30–40% heterogamic matings if the females were devoid of antennae!

LITERATURE CITED

DOBZHANSKY, TH., L. EHRMAN, AND O. PAVLOVSKY. 1957. *D. insularis,* a new sibling species of the willistoni group. Univ. Texas Publ., Genetics of Drosophila, #5721: 39–47.

MAYR, E. 1950. The role of the antennae in the mating behavior of female *Drosophila*. EVOL., **4**: 149–154.

SPIETH, H. T. 1952. Mating behavior within the genus *Drosophila* (Diptera). Bull. Amer. Mus. Nat. Hist.. **99**: 401–474.

SOME COMMENTS ON THE EVOLUTION OF THE ARTHROPODA

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The much-debated problem of the evolution of the major groups of arthropods has recently been reviewed in a detailed and scholarly manner by Tiegs and Manton (1958). The late Professor O. W. Tiegs, of the University of Melbourne, is well known for his classic studies of the embryology of the Symphyla and Pauropoda, as well as many other works, including a paper on the origin of the insects (1949). Upon his death he left the first draft of a re-

view of arthropod evolution, and this has been completed and extended by S. M. Manton, of the University of London and the British Museum, herself the author of many excellent papers on the embryology, development, and locomotory behavior of various arthropods, most particularly *Peripatus* and its allies. These authors are to be commended for bringing together in this study not only a large amount of data from their own particular fields, but also a considerable amount of pertinent information on fossil and relict arthropods. Their bibliography includes over 300 titles.

The authors begin their paper, appropriately, with the statement that "apart from the general thesis that the arthropod phylum has sprung from the annelid worms there are few points relating to its ancestry on which any real agreement has yet been reached." Yet even the annelid origin of the arthropods does not escape serious questioning as their argument develops. They remark that the group "is further removed from the Annelida than are the Mollusca," and suggest in their final sentence that "the Arthropoda may not have evolved from the Annelida but from a Pre-Cambrian group which has left no traces."

The arthropods are seen as "a most unusual instance of a major animal assemblage arising from a parental group by a process that is little more than specialization." This meaning of the word specialization seems to differ from any of the definitions of this much-abused word given by Simpson (1953), who considers that it implies either (a) a narrowing in the adaptive zone of a group, (b) a limitation of further change, or (c) a comparatively large change in a single character, such as size. The type of specialization meant by Tiegs and Manton "confers so many advantages the condition for almost unlimited convergence arises." They consider the possibility that not only the tracheal system and malpighian tubules have arisen two or more times independently in the arthropods (as most would agree), but also the compound eyes, the mandibles, the haemocoele, and even "arthropodization" itself (i.e., the development of a jointed exoskeleton). Thus, they say, the Arthropoda may well be diphyletic or even polyphyletic.

The review of the structure of the trilobites, the mid-Cambrian "Pseudo-crustacea," and the fossil chelicerates is admirable. The authors do not claim that they are able to reconstruct the phylogeny of these groups, but they consider it likely that the trilobites and the Chelicerata had a common origin in the Pre-Cambrian and that the Crustacea may also have a distant relationship to the trilobite-chelicerate stem. The Pycnogonida and Tardigrada, however, are difficult to accommodate in the scheme of evolution of the chelicerates, and may have evolved independently from Protonychophora.

The authors review the evidence that the insects arose from myriapod stock and find it convincing. They suggest that the ancestral myriapods may have developed directly (without anamorphosis) and were opisthogoneate. The development of anamorphosis in one or more lines of ascent made it expedient to develop an anterior gonopore, shown by Tiegs to be an epidermal invagination developed during the larval stage. These ideas have been developed by both Tiegs and Manton in earlier papers. The idea that the insects evolved from myriapods is now almost universally accepted, and as a result of Tiegs' work the anterior gonopore of the Symphyla no longer seems to provide the stumbling-block it once did.

The myriapod-insect stock, they believe, cannot have been derived from the Crustacea or the Trilobita. Their reasons for believing this are not developed in detail, but they have to do with complete absence of biramous appendages and second antennae in the myriapod-insect line, the "basically different" mandibles, and the lack of suitable connecting fossils. It would, indeed, be convenient for adherents of an opposite view if a Devonian myriapod possessing partially biramous appendages could be found, but the loss of the branchial arms of the appendages may have occurred very quickly when these arthropods invaded the land. It is possible that the labrum of the myriapods and insects is formed from the same lobes which give rise to second antennae in Crustacea (Butt, 1957). And whether or not the mandibles of Crustacea and Myriapod-Insecta are basically different is certainly arguable. Tiegs and Manton have little patience with the theory that the insects arose directly from Crustacea (perhaps with justification), but there are more facts to the case than simply the "superficial similarity between the amphipod-isopod head and that of insects." The papers of Crampton (1921 and others) on this subject are not mentioned or listed in the bibliography.

Having reasoned away the trilobites and Crustacea from the ancestral stock of the myriapods and insects, the authors are left with "as a possible progenitor some ancient *Peripatus*-like creature." Certain resemblances between *Peripatus* and the myriapods are pointed out; these pertain to locomotory gaits, exsertile vesicles, similarities in the mid-gut and genital tubes, and certain embryological similarities. The many remarkable differences between these animals are not enumerated in detail. Perhaps these are not important, since, according to the authors' theory of unlimited convergence, even "arthropodization" may have been acquired several times independently. Presumably the rather limited and unspectacular resemblances between

Peripatus and the myriapods are immune to this interpretation.

I do not mean to imply that the evolution of the myriapods from primitive crustaceiform arthropods, and these in turn from pro-trilobites, is at all fully documented, but at least the Crustacea have mandibles and maxillae which are regarded as homologs of those in the myriapods. The mandibular and maxillary segments in *Peripatus* do not even exist as such, but are still part of the trunk (the "jaws" of *Peripatus* have no homolog in true arthropods, unless it is the labrum or second antennae). The leg segments of trilobites, Crustacea, and other true arthropods may or may not correspond exactly, but at least the arthropod limb is quite another matter from that of *Peripatus*. I remain unconvinced that *Peripatus* is anything more than a fascinating relict and that the phylum Arthropoda is anything less than a monophyletic assemblage which arose from an annelid ancestor not by "specialization" but by progressive evolution no different from that which has characterized most major groups of animals.

LITERATURE CITED

BUTT, F. H. 1957. The role of the premandibular or intercalary segment in head segmentation of insects and other arthropods. Trans. Amer. Ent. Soc., **83**: 1–30.

CRAMPTON, G. C. 1921. The phylogenetic origin of the mandibles of insects and their arthropodian relatives—a contribution to the study of the evolution of the Arthropoda. Jour. N. Y. Ent. Soc., **29**: 63–100.

SIMPSON, G. G. 1953. The major features of evolution. New York, Columbia University Press.

TIEGS, O. W. 1949. The problem of the origin of insects. Australian and New Zealand Assoc. Adv. Sci., Report 27th Meet. Sect. D, pp. 47–56.

——, AND S. M. MANTON. 1958. The evolution of the Arthropoda. Biol. Rev. Cambridge Philos. Soc., **33**: 255–337.

PERSPECTIVES IN MARINE BIOLOGY[1]

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In the spring of 1956, a symposium on perspectives in marine biology was held at Scripps Institution of Oceanography. One of the products of the symposium was the recent publication of the papers presented at the meeting together with the discussions by the participants. The forty-one contributors to this volume nearly form a world's Who's Who in marine biology. Notably absent from the meeting, however, were the very active Russian workers in this field.

The purpose of the symposium as stated by the editor was "to permit a discussion focused on possible forthcoming fields of development in marine biology rather than a survey of past accomplishments." The result of the symposium, at least as reflected in this book, was a very useful and cogent statement of current research. As might have been expected, most of the forthcoming fields of development in marine biology are developing right this moment and in the very laboratories of the participants.

The volume is organized into four parts: Ecology, Physiology and Biochemistry, Behavior, Genetics and Evolution. Under these headings very diverse subjects are discussed, ranging from the techniques of underwater television to evolution in the microcosmos of the Chemostat. The papers on genetics and evolution of marine organisms bring together, for the first time, the scant zoological literature in this field. The discussions in this section serve to emphasize how little is known concerning speciation in the sea. The volume should be of interest to all biologists and serve as a fitting companion to the Geological Society of America's *Treatise on Marine Ecology* and Moore's recent *Marine Ecology*.

[1] Perspectives in Marine Biology edited by A. A. Buzzati-Traverso. University of California Press, 1958: 1–621 p., $10.00.

ANNOUNCEMENTS

The Karl P. Schmidt Fund has modest sums available to assist persons wishing to study at the Chicago Natural History Museum. Grants will be made for study in any of the four fields encompassed by the museum: anthropology (with a natural history orientation), botany, geology (including paleontology), and zoology. An applicant should describe the study proposed in brief terms, state the length of time he wishes to study at the museum and the amount of money needed, and name one reference.

Applications should be mailed to: Chairman, The Karl P. Schmidt Fund, c/o Chicago Natural History Museum, Roosevelt Road and Lake Shore Drive, Chicago 5, Illinois.

The Division of Biological and Medical Sciences of the National Science Foundation announces that the next closing date for receipt of basic research proposals in the life sciences is May 15, 1959. Proposals received prior to that date will be reviewed at the summer meetings of the Foundation's advisory panels and disposition will be made approximately four months following the closing date. Proposals received after the May 15, 1959, closing date will be reviewed following the fall closing date of September 15, 1959.

Inquiries should be addressed to the National Science Foundation, Washington 25, D. C.